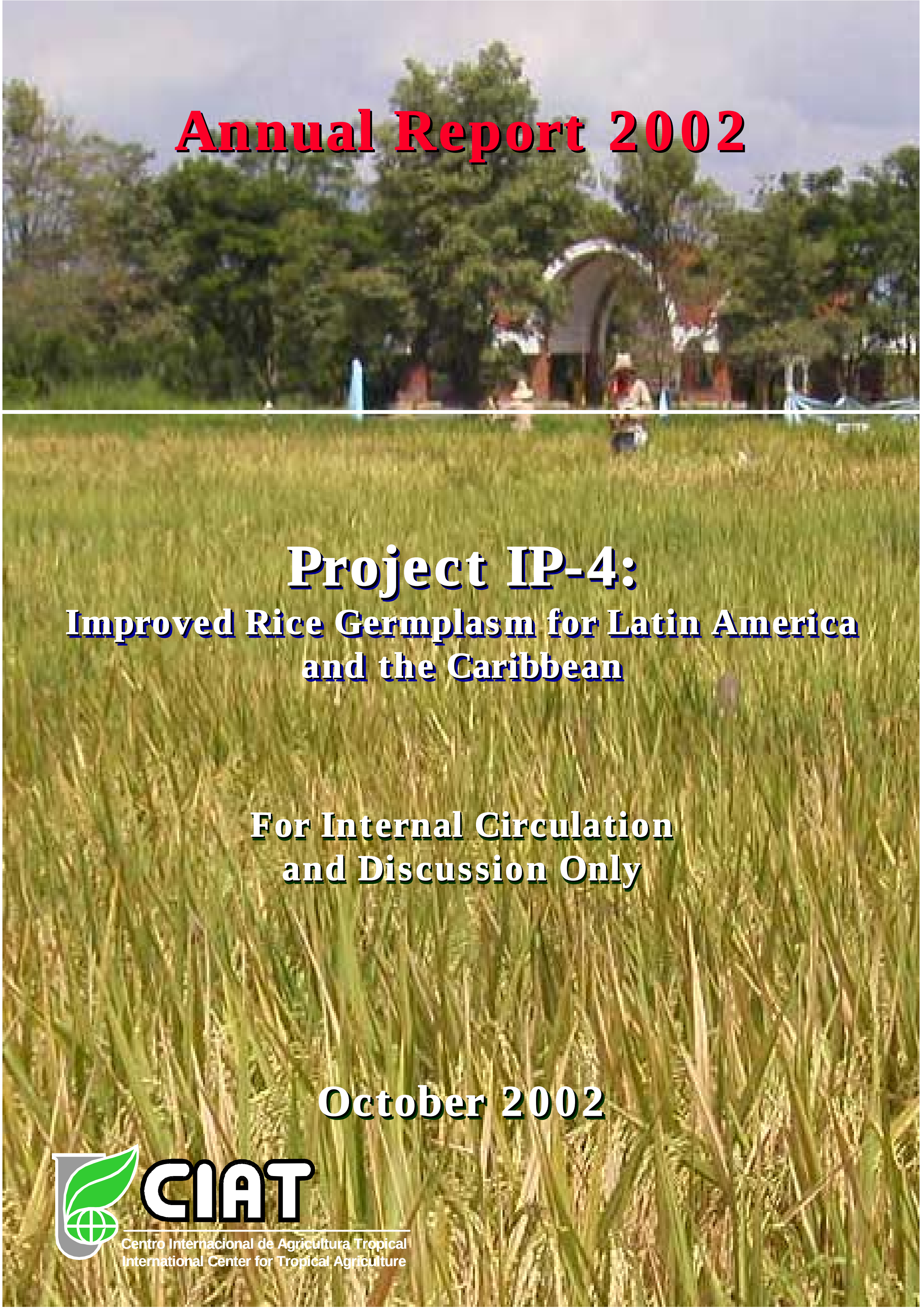

Annual Report 2002

Project IP-4:

Improved Rice Germplasm for Latin America and the Caribbean

For Internal Circulation
and Discussion Only

October 2002

CIAT
Centro Internacional de Agricultura Tropical
International Center for Tropical Agriculture

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Executive Summary

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

Project Description

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

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

Gains: Broader genetic base available and germplasm better characterized. New sources of resistance to diseases, viruses, and insects incorporated and made available. Higher yielding, advanced, rice lines. Variability and stability of progenitors and of advanced materials made available to increase breeding efforts. Rational pesticide use with fewer environmental hazards. Lower unit costs conductive to higher profits and lower rice prices to consumers.

Milestones:
2002 Molecular markers associated with blast resistance genes identified and used in marker-assisted selection. Sources of blast resistance distributed to national breeding programs. Improved rice populations with broader genetic base developed by recurrent selection and distributed to national programs in LAC. Rice cultivars released for savannas, lowland and highlands ecosystems. Participatory breeding strategies began to be implemented. Epidemiological studies for the control of RHBV and its vector, *T. orizicolus* completed. Potential use of transgenic plants with resistance to RHBV evaluated in the field. Rice germplasm with improved grain quality and milling developed together with FLAR. National scientists from LAC trained in new technologies used at CIAT.
2003 Improved rice cultivars using wild rice genes and recurrent selection populations. Introgression of new plant type (IRRI) into LAC's gene pools. Evaluation and selection of improved rice populations with broader genetic base by national programs in LAC. Characterization of rice blast pathogen populations in LAC. Identification of relevant blast resistance genes for LAC blast populations. Promotion of IPM strategies for controlling RHBV and *T. orizicolus.* RHBV-viral genes from transgenic plant introgressed into commercial rice cultivars. Rice germplasm with improved grain quality and milling developed together with FLAR. Selection of rice lines with tolerance to submergence for an improved weed control strategy. Functional genomics is being started in collaboration with GENOPLANTE.
2004 Genetic progress and gains in recurrent selection for different traits will be assessed in several LAC countries. Near-isogenic lines with QTLs associated with yield developed for use in LAC breeding programs. Genetic gains for yield derived from interspecific crosses will be evaluated after introgression of wild genes into cultivated LAC rice varieties. Implementation of breeding methods for durable blast resistance in LAC based on population dynamics of pathogen populations and partial resistance. Molecular and virulence characterization of other rice pathogens. Management of RHBV and its vector based on epidemiological studies. Commercial rice cultivars with transgenes for RHBV tested in LAC. Participatory rice selection and breeding will be producing new rice varieties for resource poor farmers.

Users: Breeders throughout Latin America and available elsewhere. Ultimate beneficiaries are poor urban consumers and rice farmers.

Collaborators: CIRAD, IRD, FLAR (Fund for Latin American and Caribbean Irrigated Rice), IRRI, WARDA. NARS (e.g., EMBRAPA, CORPOICA, IDIAF, INIAP, INIA, IIA), U.S. Universities (Cornell, Purdue, LSU, Arkansas, Texas A&M, California, Florida State, Yale), JIRCAS, GENOPLANTE.

CGIAR system linkages: Enhancement and Breeding (50%); Crop Systems (5)%; Protecting the Environment (15%); Saving Biodiversity (20%); Strengthening NARS (10%). Linked to IRRI global rice research and WARDA interspecific crosses.

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

Project IP-4 Log-Frame 2002.
Improved Rice Germplasm for Latin America and the Caribbean

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

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

Summary of Annual Report 2002

Inputs

Principal Staff	Allocation of time	Affiliations	Work Location
Dr. Lee Calvert	60%	CIAT	CIAT HQ
Dr. Marc Chatel	100%	CIRAD/CIAT	CIAT HQ
Dr. Fernando Correa	100%	CIAT	CIAT HQ
Dr. Zaida Lentini	20%	CIAT	CIAT HQ
Dr. Mathias Lorieux	50%	IRD/CIAT	CIAT HQ
Dr. César Martínez	51%	CIAT	CIAT HQ
Dr. Rafael Meneses	50%	IIA Cuba/CIAT	CIAT/Cuba
Dr. Gilles Trouche	50%	CIRAD/CIAT	Managua, Nicaragua
Dr. Michel Valès	100%	CIRAD/CIAT	CIAT HQ
Total	5.81 Principal Staff positions		
Dr. Carlos Bruzzone works as a consultant	50%	CIAT	Chiclayo, Peru

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

Principal Cooperators by Organization
U. of Tucumán & U. La Plata Argentina, CIAT Santa Cruz Bolivia, EMBRAPA Brazil, INIA Chile, FEDEARROZ & CORPOICA Colombia, IIA Cuba, IDIAF Dominican Republic, CIRAD & IRD France, DICTA Honduras, JIRCAS, Japan, INTA Nicaragua, INIA Peru, INIA Uruguay, Cornell, UC at Davis, KSU & LSU, Texas A&M USA, INIA & DANAC Venezuela, IRRI, and WARDA, and FLAR.

Highlights

Rice, a crop with impact

In output 1A, there is the section "Impact in Latin America of CIRAD's Rice Genetic Resources". This documents the successful use of these genetic resources as the parents of varieties. Over 90% of the approximately 40 upland varieties released in the last 20 years have at least one CIRAD parent. Germplasm that has been developed by CIAT has also been crucial as parents, and often the crosses for these varieties were made at CIAT. For the last decade, CIRAD and CIAT have had a strategic alliance that has worked synergistically by bring the critical human capital, resources and infrastructure together to help serve the Latin American and Caribbean rice sectors. The effectiveness of this alliance depends on our national partners. An excellent example, where CIAT, CIRAD, CORPOICA, and FEDEARROZ worked together, is documented in the impact study "Un negocio de amplios horizontes para el Llano". It is estimated that the economic impact of CIAT rice projects contribution is $450 million between 1994-2001. The strategic alliance of CIRAD/CIAT with many local partners will continue to have lasting impact in the Llanos of Colombia and throughout Latin America.

The first rice variety that was developed using recurrent selection in Latin America

Using recurrent selection for rice breeding has been somewhat controversial. Nevertheless, the alliance of CIAT and CIRAD continues to promote this method. The EMBRAPA and CIRAD collaborative project in Brazil is now bearing fruit. In the State of St. Catarina Brazil, a new variety named Tio Taka was released in 2002, and it was produced using recurrent selection. There are currently populations that have been developed using recurrent selection in eleven countries, and there are many advanced lines that could soon be selected as new varieties. We expect that Tio Taka will be the first of many varieties that have their origins in recurrent selection populations, and this shows the importance of maintaining a consistent effort over a sufficient period of time.

Using wild rice species to improve cultivated rice in Latin America

The genera Oryza possess very high genetic diversity and 21 wild rice and two cultivated species are known. After 6,000 years of continuous selection by man and intensive breeding efforts during the last 100 years, the genetic base of the crop is narrower than ever. Modern rice varieties that ushered in the green revolution brought about dramatic increases in rice production worldwide, but a narrower genetic base. There are many reports of rice production in farmers' fields reaching a yield plateau, and the narrow base is contributing to instability of rice yields caused by biotic and abiotic factors. The Oryza wild species represent a potential source of new alleles for improving the yield, quality and stress resistance of cultivated rice. Results presented in this report and generated in collaboration with key partners provide further evidence that certain regions in *O. rufipogon* and *O. glaberrima* harbor alleles of interest for the genetic improvement of cultivated rice. In the interspecific cross of Bg90/*O. rufipogon*, selected progeny yield better than either parent suggest the greater yields can be achieve through the introgression of alleles from wild species. Even more surprising is despite the poor grain quality of both parents Bg90-2 and *O. rufipogon*, through positive transgressive segregation, we were able to select advanced lines with long and slender, translucent grains. The results for disease resistance are also impressive. Using high disease pressure, advanced breeding lines with resistance to several fungal diseases particularly to *Rhizoctonia solani* were derived from the interspecific crosses of Oryzica3/*O. rufipogon*. High level of resistance to the rice stripe necrosis virus was found in *O. glaberrima*, and this resistance has been transferred through interspecific crosses to Bg90-2 and Caiapo. Advanced interspecific lines that have been tested in farmer's fields confirm that *O. rufipogon* and *O. glaberrima* possess alleles with positive effects on yield, stress resistance and grain quality. Molecular markers are being used to map QTLs associated with these traits and near isogenic lines are being developed for use in breeding programs. We are using the wild species to increase genetic diversity while developing varieties with high and stable yields.

The long road to developing durable rice blast resistance

There are voices of pessimism that lament the lack of progress on developing rice with durable resistance to rice blast. There is no doubt that the fungus has a chimerical ability to breakdown resistance and to remain the most important disease of rice. There are molecular studies that are identifying the resistant genes, and others that are bringing order to the many races of the fungi. These studies will be the basis to develop a durable resistance in a systematic manner that targets specific combinations of genes. Even as this research is progressing, there is a tendency to produce varieties that remain resistant to rice blast for longer periods of time. For example, the variety Fedearroz 50 is widely grown in Colombia and has remained highly resistant to rice blast for over three years. The genetic resistance profile of Fedearroz 50 is similar to that of Oryzica Llanos 5, which has remained resistant to rice blast for over 12 years. This contrasts with many varieties that start to have problems one to two years after their release. Much work remains before we can declare that we have the knowledge and methods to consistently develop rice with durable resistance, but there is evidence that step-by-step, we are making progress.

The new rice hoja blanca resistant varieties are better than their parents

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

Are the small rice farmers doomed to subsistence living?

In Latin America, rice is considered by many to be a crop for the large farmer. It takes lots of infrastructure such as irrigation and leveled fields, while the price is relatively low, therefore the small rice farmer cannot make a decent living. The highly mechanized farms are only one part of the rice sector in Latin America. Most rice farmers are small and grow rice in favored upland conditions, and they do need help if they are going to get out of the poverty trap. The CIAT/CIRAD alliance shares a vision to help these farmers by producing varieties that produce higher yields in upland conditions. This year, we expanded our activities in Central America. For several years, we have been working in Colombia and these efforts are beginning to have impact with the development of the first varieties of the new type Rice for Hillsides with Cold tolerance "RHICO". Also the rice sector on the Pacific coast is being reactivated with the introduction of seeds from commercial varieties and an effort to develop varieties that are specifically adapted to the local conditions. To be successful, the local farmers need high yields and a way to market their surplus rice. We also need to develop a strategy that generates additional employment both for small farm equipment as well as the processing and marketing of rice. Further, rice should be a stable component in the cropping system that allows these small farmers the ability to take risks with higher value crops. Rice can be a motor that drives these small farmers into a more prosperous life. We need the vision to help them make it a reality.

Plans for next year

- The breeder's workshop for irrigated rice will be held in the Dominican Republic and for upland rice in Colombia.
- The launching of International observational nurseries (CIAT-ION) for irrigated and upland rice.
- Developing an alliance with the Rural Innovation Institute of CIAT to develop participatory projects on the chain of rice production in agricultural systems.
- The use of anther culture, transgenics, and molecular markers as advanced breeding tools for rice
- Continuing to find and incorporate better resistance for major pests and diseases into CIAT populations and advanced lines.
- Incorporating additional traits from wild relatives of rice.
- Developing more drought resistant upland rice with partners in CIRAD, IRD, EMBRAPA and WARDA.

Project Performance Indicators

1. Technologies, Methods and Tools

1.1 Released Varieties

The crosses for most of these varieties were made at CIAT. Details on the date and parents of the crosses are available upon request.

Bolivia	Tacuú by CIAT-SC	2002
Brazil	BRS Talento by EMBRAPA	2002
	BRS Jaqiaru by EMBRAPA	2002
	Tio Taka by EPAGRI	2002
Dominican Republic	INIAP-1 by INIAP	2002
Nicaragua	INTA 2000 by INTA	2002
Venezuela	D-Sativa by DANAC	2002

1.2 Genetic Materials Distributed

- Breeding nurseries made up of advanced lines derived from interspecific crosses were sent to partners in Colombia, Argentina, Uruguay, Venezuela, Suriname, Nicaragua, and Peru
- 400 progenitors of CIRAD-Centre Francais du riz (CFR) were provided to FEDEARROZ (Colombia), CIAT-Bolivia, and DANAC (Venezuela)
- 323 CIAT progenies from CIAT/Peru program and from interspecific crosses *Oryza sativa* x *O. glaberrima*, *O. sativa x O. barthii* and *O. sativa* x *O. rufipogon* for lowland irrigated conditions or favorable aerobic conditions (from Cesar Martinez program)
- 24 CIAT-CIRAD progenies derived from PCT-4 population for aerobic upland conditions (from Marc Châtel program)
- 5 CIAT-CIRAD varieties for upland conditions: CIRAD 409, 446, 447, ORYZICA Sabana 6, ORYZICA Sabana 10
- 6 CIAT varieties for irrigated conditions: Fedearroz 50, Fedearroz 2000, Bg90-2, Oryzica 1, Selecta 320 and Oryzica 3

Participatory Breeding

- PCT-18: narrow genetic base population adapted for upland conditions for rice blast resistance and grain quality (from Michel Vales program)
- PCT-17: narrow genetic base population adapted for high altitude hillsides upland conditions for rice blast resistance, cold tolerance and grain quality (from Michel Vales program)

1.3 Elite Materials Developed

- Advanced lines with tolerance to Rhizoctonia solani and the rice stripe necrosis virus have been developed.
- The first upland varieties for hillsides with tolerance to drought and to cold, and with high level of partial resistance to rice blast disease are nominated as a results of a participatory evaluation: CIRAD 446 and CIRAD 447. They are the first

varieties of RHICO type (Rice for Hillsides with Cold tolerance) in Latin America.
- The upland savanna line CT10069-27-3-1-4 (CIRAD/CIAT 445) is adapted to inter-cropping with young coffee plantations in Colombia, and the CIAT upland line CT13226-11-1-M-BR1 will be released in 2003 in Brazil.
- An elite upland line (PCT-4\SA\1\1>975-M-2-M-3) developed in Colombia from recurrent selection breeding

Participatory Varietal Selection

- 14 advanced lines and varieties from INTA Rice Program for upland and lowland conditions
- 9 CIRAD varieties from collaborative program in Ivory Coast, Brazil and Madagascar for less favorable upland conditions
- 14 CIAT-CIRAD advanced lines for high altitude hillsides

1.4 Genetic Mechanisms Understood

There is a strong correlation between RHBV titer in the propagative vector *T. orizicolus* and its ability to transmit the virus.

1.5 Sources Identified

Oryza rufipogon (IRGC105491) has been showed to have a good level of tolerance to *Rhizoctonia solani* under greenhouse and field conditions.

2. Publications

2.1 Referred Journals

Published

Two articles were published and one article has been accepted for publication.

Submitted

One article has been submitted for publication.

2.2 Books

Book Chapters

Three book chapters were published.

Published Proceedings

Four articles were published in Proceedings.

Scientific Meetings or Publications

Ten abstracts were published and presented at scientific meetings.

3. Strengthening NARs and NGOs

3.1 Individual Training

The rice project worked with 9 scientists for intensive specialized training.

3.2 PhDs, MS & BS

The rice project is involved in the thesis with 7 Bachelor of Sciences, 10 Masters of Science and 1 PhD students.

3.3 Workshop and Meetings

The rice project sponsored or participated in 10 workshops that had over 300 participants.

3.4 Technical Assistance

CIAT provided technical assistant to FLAR in the areas of rice blast screening in the southern cone.

Technical assistance was given to many programs on breeding activities.

3.5 Advanced Research Organizations Research Partnerships

The CIAT rice project maintains research partnerships with CIRAD and IRD in France, JIRCAS in Japan, and with Cornell, UC at Davis, Kansas State, Louisiana State, and Texas A&M in the USA. We also collaborate with WARDA and IRRI.

4. Impact Monitored

An impact study for the Convenio Colombia-CIAT was made. It is titled “Un negocio de amplios horizontes para el Llano”. It estimated that the new rice varieties that were developed in collaboration with the rice project have had an impact of $450 million in just the Llanos of Colombia during 1994-2001.

The section 1A of the Annual Report IP-4 2002, documents use of CIRAD germplasm in upland rice. Of the 40 varieties released in the past 20 years, more than 90% had CIRAD parents in their background.

CIAT
Centro Internacional de Agricultura Tropical
International Center for Tropical Agriculture

Improved Rice Germplasm for Latin America and the Caribbean

Recognitions received during 2002

Brazil

Brazil's Rice Production Chain made a public recognition of the contributions made by CIAT's Rice Project toward the development of irrigated and upland rice cultivars in that country over the past 20 years. The award was presented during the inauguration ceremony of the First Brazilian Rice Production Chain Congress, held between 20 and 23 August 2002, at the Convention Hall in Florianópolis (Santa Catarina, Brazil).

Dominican Republic

On 7 July 2002, the National Cereal Grains Program of the Dominican Agricultural and Forest Research Institute, IDIAF, presented a plaque of recognition to César Martínez, CIAT Rice Project Breeder, within the framework of the release of a new rice variety, IDIAF 1, and the celebration of the 40th anniversary of the Juma Experiment Station, events that were attended by the President of Dominican Republic, Hipólito Mejía.

César Moquete, Head of the National Cereal Grains Program, stated that this recognition was given for the valuable contributions made by Martínez to the genetic improvement of rice in Latin America, enabling the expansion of the genetic base of this grain, and his contribution to the training of many of the region's rice researchers.

Bolivia

Within the framework of the 4th National Rice Day, held in Santa Cruz, Bolivia, rice breeder Marc Chatel from CIRAD/CIAT received a plaque from Bolivia's Tropical Agricultural Research Center in recognition of "his support and collaboration in training, germplasm supply, breeding methodologies, and unconditional friendship to CIAT-Bolivia and the Santa Cruz rice sector", signed by the San Juan Japanese Colony on 9 March 2002.

OUTPUT 1. ENHANCING GENE POOLS

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

• Impact in Latin America of CIRAD's Rice Genetic Resources

Marc Châtel and Elcio Guimarães

Abstract

Increased production of rice has led to a lower price of rice for the consumers. Since rice is a staple food and principal source of calorie for the 20% lower-income people of Latin America and the Caribbean (LAC), this has benefit the poor throughout the region. The adaptation of the Asian green revolution to LAC and the release and adoption of high yielding varieties (HYV) was responsible for the increase of rice production in the region. During the last 40 years, about 300 varieties were released (262 and 37 for irrigated and upland respectively). They were developed by (i) the International Rice Research Institute (IRRI), (ii) the Centro Internacional de Agricultura Tropical (CIAT), (iii) the national rice research programs and (iv) the collaborative research projects between the Centre de Coopération Internationale en Recherché Agronomique pour le Développement (CIRAD France) and Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA Brazil) and CIAT Colombia, (Upland rice). The regional networks like INGER-LAC and FLAR, as well as bilateral collaborations are important for the diffusion of the new HYV.

This report analyses the contribution of the genetic resources of CIRAD in the background of the varieties released. For aerobic upland rice, 90% of the varieties have one or more CIRAD parent. In LAC, the genetic resources of CIRAD represent a great contribution, but only few CIRAD lines were repeatedly used as parent (CIRAD 2, CIRAD 120 series and CIRAD 216). This leads to a possible narrowing of the genetic base. Furthermore, the new released varieties were used as parent for new crosses leading to a higher degree of consanguinity. Facing this problem, the rice project of CIAT focused on pre-breeding activities aimed at broadening the genetic base of rice in LAC.

Keywords: Rice, genetic resources, high yielding varieties, and Latin America and the Caribbean

Introduction

In LAC, since starting in the 60's, CIAT has been a leading research institution that have developed and contributed to the released new HYV in the region. CIAT started introducing rice germplasm from IRRI (Asian green revolution) to identify adaptation to the rice ecosystem of LAC and parents for crossbreeding. Later on, the introduction of germplasm was directed towards the specific needs of the different rice ecosystems (Irrigated tropical, sub-tropical and temperate climate, and aerobic upland rice for the savannas). With the set-up of the regional rice project mandate and the increase in breeding activities, many breeding lines were developed and shipped to the national breeding programs through the INGER-LAC network, for local evaluation and possible release. The main constraints were addressed by identifying parents with biotic and abiotic traits of resistance, and using these in breeding activities. During the last 30 years, about 300 varieties were released as cultivars in LAC. A great majority come from progenies of crosses developed and selected by CIAT.

CIRAD's Rice Genetic Resources in LAC

Until the mid 80's, CIAT concentrated on breeding to the irrigated rice sector of LAC, while starting in the 60's, CIRAD developed rice breeding for the upland rice ecosystem present in the West African countries as well as activities in irrigated ecosystem like Madagascar. In the 80's, CIRAD started rice breeding activities in Latin America, mainly in Brazil and French Guyana. Since 1992, CIRAD and CIAT have maintained an alliance to develop rice lines together. The genetic resources developed by CIRAD were shared with CGIAR Research Centers working in rice including CIAT. Thanks to the CIAT's rice data bank and the different publications of the institution, it has been possible to collect and analyses the information about the use of CIRAD's genetic resources in LAC. The present report analyses the contribution of the CIRAD's rice genetic resources in different breeding programs, as well as for their genetic contribution in the varieties released by LAC-NARS.

Use of the CIRAD Rice Genetic Resources by LAC NARS

The CIRAD varieties introduced by CIAT through the global IRTP and INGER Networks or directly through bilateral collaborations between CIRAD and LAC-NARS, were characterized and used as potential parents by the rice breeding programs in different countries (Table 1).

Brazil: In Brazil, three public research centers are working in rice breeding: EMBRAPA Rice and Beans Center (CNPAF), State of Goiàs, IAC Campinas-Research Center of the State São Paulo and EMBRAPA Lowland Center-State of Rio Grande do Sul. A total of 53 CIRAD rice varieties were used as parents in crosses by the three institutions. EMBRAPA Rice and Beans Center used 50 in 511 crosses, 284 times as female and 227 times as male parent. These numbers represent very well the intensity of the direct collaboration established during the period 1981-1991, in the framework of the upland rice-breeding project between EMBRAPA and CIRAD. Eight of these 50 varieties selected by CNPAF were used by IAC in 77 crosses, 10 times as female and 67 times as male parent. The lowland Center of EMBRAPA used only three CIRAD varieties, of which 2 were different from the ones used by the other Brazilian centers. This resulted in fifteen crosses always using the CIRAD variety as the male parent. In this last case, the CIRAD varieties were not used frequently because the lowland EMBRAPA Center focuses its breeding program on sub-tropical and temperate climate irrigated lowland rice ecosystem while the CIRAD varieties are adapted to both upland and lowland tropical rice ecosystems. The most successful CIRAD varieties used as parents in Brazil were CIRAD 2, CIRAD 13, CIRAD 101, CIRAD 112, CIRAD 177, CIRAD 195, CIRAD 216 and CIRAD 257. They are upland materials with high yield potential, good plant type, earliness, and one of them (CIRAD 216) has good grain quality (long slender grain).

Colombia: In Colombia, two research institutions conducted rice breeding. They are the public Instituto Colombiano Agropecuario (ICA), and the international institution CIAT. During the period 1967-1983, the two institutes collaborated to design and made crosses, then CIAT took-over the responsibility. A total of 16 CIRAD varieties were used in 1092 crosses, 703 times as female and 389 times as male parents. The most successful CIRAD varieties used as parents in Colombia were CIRAD 8, CIRAD 13, CIRAD 120, CIRAD 121, CIRAD 122, CIRAD 124 and CIRAD 216. The varieties of the series CIRAD 120 bred in Madagascar were used intensively. These varieties were identified as source of resistance to the rice hoja blanca virus (RHBV). As mentioned earlier they were bred in Madagascar, were the RHBV does not exit. Looking at their genetic constitution, they have the traditional line Makalioka 34, cultivated in the Alaotra lake region, as one of their parents. Makalioka 34 was identified by CIAT as a source of resistance to the RHBV.

Mexico: In Mexico, the Instituto Nacional de Investigaciones Forestales y Agropecuarias (INIFAP) used 17 CIRAD varieties in 277 crosses.

Peru: In Peru, the Instituto Nacional de Investigaciones Agropecuarias y Agroindustriales (INIAA) used 8 CIRAD varieties in 74 crosses.

Ecuador: In Ecuador, 3 CIRAD varieties were used in 15 crosses.

Release of Varieties Selected from Crosses with CIRAD Germplasm

The CIRAD germplasm used by the national rice breeding programs, have permitted the development of promising progenies from which some were released as commercial varieties (Table 1). The most relevant impact, during the period 1981-1991, was in Brazil in the framework of the collaborative project between EMBRAPA-CNPAF and CIRAD. Sustainable impact in this country continues through the collaborative rice breeding project between CIAT, CIRAD, CNPAF with emphasis in upland rice breeding.

Brazil: During the period 1986–2002, fourteen upland savanna lines, bred by the EMRAPA/CIRAD and CIAT/CIRAD collaborative rice breeding projects, were released.

EMBRAPA/CIRAD lines: One of the outputs of rice breeding project between EMBRAPA Rice and Beans Center and CIRAD (1981–1991) was the release and adoption by farmers of eight modern upland lines for the savannas ecosystem of the Brazilian Cerrado. These varieties are RIO PARANAIBA, CENTRO AMERICA, GUARANI, GUAPORÉ, TANGARA, DOURADÃO, XINGU and PRIMAVERA

CIAT/CIRAD lines: During the period 1992-2002, 67% of the upland rice lines released in Brazil came from CIAT. They were selected in Colombia, by the CIAT/CIRAD collaborative rice-breeding project, and then adapted by EMBRAPA-CNPAF. The following varieties have at least one CIRAD parent: PROGRESO, CANASTRA, MARAVILHA, BONANÇA, CARISMA and TALENTO.

Colombia

CIAT and CIAT/CIRAD lines: During the period 1989–2002, five lines having at least one CIRAD parent, were released as cultivars. Two are from CIAT for the irrigated lowland ecosystem, three are from CIAT/CIRAD for upland savannas ecosystem. The varieties are ORYZICA SABANA 6, ORYZICA SABANA 10, ORYZICA LLANOS 4, PROGRESO 4-25 and CIRAD/CIAT 409.

Bolivia

CIAT lines: Three lines were released for the favorable upland rice ecosystem. The varieties that are from CIAT and have at least one CIRAD parent are SACIA-1 (TACU), SACIA-3 (TUTUMA) and SACIA-4 (JISUMU).

Guatemala

CIAT lines: Three lines were released. They are from CIAT and have at least one CIRAD parent: ICTA IZABAL, MASAGUA and OASIS.

Direct Release of CIRAD Varieties

Five varieties from CIRAD showing good direct adaptation were released as commercial varieties in Brazil and Bolivia. In Brazil the varieties are IRAT 190, IRAT 177 and IRAT 216, and in Bolivia they are IRAT 170 and IRAT 357 (Table 2).

CIRAD/CIAT Promising Lines

Colombia

Six lines have been identified as very promising. Three in Colombian, one in Peru, one in Brazil and one in Nicaragua (Table 3).

Conclusion

In Latin America, the impact of the rice genetic resources of CIRAD is high. Thirty-one new lines were released, mainly for the upland savannas, although some are for the irrigated and hillside rice ecosystems. Three new lines (CIRAD/CIAT 4445, CIRAD/CIAT 446 and CIRAD/CIAT 447) were identified as very promising for the Colombian hillsides ecosystem.

The impact was very important in Brazil, Bolivia and Colombia, and this is related to two principal factors. The first one is the rice strategy that CIRAD implemented in the 60’s, when the institution started working in upland savannas breeding for West Africa. A great number of enhanced lines were developed to attend the main rice ecosystem present in the region. The second factor was the development of bilateral and international rice breeding projects in Latin America with EMBRAPA-Brazil and CIAT-Colombia respectively. Doing research together has been fruitful.

Nevertheless, only few CIRAD varieties were repeatedly used as parent, and this is of concern for the genetic base of the released varieties. Furthermore, the new commercial varieties were used as parents in new crosses leading to a certain degree of inbreeding. That is why, at the beginning of the 90’s the CIAT rice project shifted its breeding strategy, and has placed more emphasis towards pre-breeding activities that enhance and broaden the genetic base of rice.

Future Activities

- Follow-up of the use of CIRAD germplasm by LAC NARs
- Monitoring the release of new varieties

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Table 1. Released Varieties Coming from Crosses with CIRAD Germplasm

Brazil - EMBRAPA/CIRAD Crosses		
Variety (Upland savannas)	**Year**	**CIRAD Parent**
Rio Paranaiba	1986	IRAT 2
Centro America	1987	IRAT 2
Guaraní	1987	IRAT 2
Guaporé	1988	IRAT 13
Tangara	1989	IRAT 13
Douradão	1989	IRAT 2
Xingu	1989	IRAT 13
Primavera	1997	IRAT 10
Brazil - CIAT/CIRAD Crosses		
Progreso	1993	IRAT 124 and IRAT 216
Canastra	1995	IRAT 122
Maravilha	1995	IRAT 121 and IRAT 216
Bonança	1999	IRAT 124 and IRAT 216
Carisma	1999	IRAT 124 and IRAT 216
Talento	2002	IRAT 124 and IRAT 216
Colombia - CIAT/CIRAD Crosses		
Variety (Upland Savannas)	**Year**	**CIRAD Parent**
Oryzica Sabana 6	1992	IRAT 216.
Oryzica Sabana 10	1996	IRAT 124 and IRAT 216.
Línea 30 (CIRAD 409)	2002	IRAT 124, IRAT 146 and IRAT 216.
Variety (Lowland Irrigated)	**Year**	**CIRAD Parent**
Oryzica Llanos 4	1989	IRAT 122.
Progreso 4-25	2000	IRAT 120
Bolivia - CIAT/CIRAD Crosses		
Variety (Favorable upland)	**Year**	**CIRAD Parent**
Sacia-1 (Tacu)	1993	IRAT 216
Sacia-3 (Tutuma)	1994	IRAT 124 and IRAT 216
Sacia-4 (Jisumu)	1994	IRAT 124 and IRAT 216
Guatemala - CIAT/CIRAD Crosses		
Variety	**Year**	**CIRAD Parent**
Icta Izabal	1996	IRAT 122 and IRAT 216
Masagua	2000	IRAT 122
Oasis	2000	IRAT 121

Table 2. Direct Release of CIRAD Germplasm

Brazil		
CIRAD Variety (Upland)	**Local Name**	**Year**
IRAT 190	Irem 16 B	1982
IRAT 177	Cabaçu	1988
IRAT 216	Rio Verde	1991
Bolivia		
CIRAD Variety (Upland small-holders)	**Local name**	**Year**
IRAT 170	Jasaye	1999
IRAT 357	Jacuú	2002

Table 3. Promising CIRAD/CIAT Lines

Colombia		
Hillsides	**Cross**	**CIRAD Parent**
CIRAD 445	CIAT/CIRAD	IRAT 104 and IRAT 216
CIRAD 446	CIRAD, selected at CIAT	IRAT 265
CIRAD 447	CIRAD, selected at CIAT	IRAT 265
Peru		
Slash and Burn	**Cross**	**CIRAD Parent**
CIRAD 409	CIAT/CIRAD	IRAT 124, IRAT 146 and IRAT 216
Brazil		
Upland Savannas	**Cross**	**CIRAD Parent**
CNA 8812 or CT13226-11-1-M-BR1	CIAT/CIRAD and Embrapa	IRAT 13, IRAT 124 and IRAT 216
Nicaragua		
Low Altitude Hillsides	**Cross**	**Year**
CIRAD 301	CIRAD	IRAT 104

- ## Conventional and Inter-Specific Cross-Breeding of Upland Rice for the Savannas Ecosystem

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

Conventional crossbreeding has permitted the development and release of modern upland lines in Bolivia, Brazil and Colombia. Although no new conventional *"Japonica" Oryza sativa* crosses have been made since 1996, we have advanced segregating lines in the pipeline and have continued their evaluation and multiplication in Colombia for use by LAC NARS. The CIAT rice project now concentrates on broadening the genetic base of upland rice. Inter-specific crosses between *Oryza sativa* and the African cultivated *Oryza glaberrima* as well as with wild relative species is one of the breeding strategies used to achieve the objective.

Keywords: Conventional cross-breeding, *Japonica Oryza sativa,* wild species, genetic base, Latin America and line release.

Introduction

The broadening of the genetic base of rice is a key element in our breeding strategy. This report focuses on the advanced segregating lines made prior to 1996, as well as the inter-specific crosses of *Oryza sativa* by *O. glaberrima*, and *Oryza sativa* by wild relative species. The evaluation and selection progenies of inter-specific crosses were developed at CIAT between *Oryza sativa*, *Oryza glaberrima* and *Oryza barthii*. We also evaluated and selected NERICA lines (NEw RICe for Africa*)* introduced from WARDA.

Material and Method

1. **Source of Germplasm:** A total of 751 lines were evaluated at La Libertad Experimental Station (LES) in Villavicencio- Meta, Colombia.
   - 30 lines from conventional crossbreeding *(Oryza sativa)*.
   - 383 lines from inter-specific crosses: 45 from *Oryza sativa* by *O. glaberrima*, 24 from *Oryza sativa* by *O. barthii* and 314 from Caiapo/ *Oryza glaberrima.*
   -338 NERICA lines introduced from WARDA and selected by CIAT.

2. **Experimental Design and Traits:** The experimental design and the traits evaluated are the same that were reported last year.

Results and Discussion

Conventional Crosses *(Oryza sativa)*: Thirty lines from conventional cross-breeding were evaluated and 27 selected and harvested in bulk, and the seed was stored. This material will be shipped to LAC NARS upon request for local evaluation and selection, through the new germplasm nursery networks (VioCIAT).

Variety Releases

Bolivia. The Centro de Investigaciones Agricola Tropical of Santa Cruz de la Sierra (CIAT Santa Cruz) and the Japanese cooperation (JICA) are working with small farmers to promote and use new upland varieties. Through the project named “Distribución de Semilla de Arroz de alta Calidad para Pequeños Agricultores” (DISAPA) they organize on-field demonstration, participatory seed multiplication and diffusion of seeds of high quality. In 2002, CIAT Santa Cruz released the line CIRAD357 as JACUÚ, for smallholders.

Brazil. During the period 1986–2002, sixteen upland lines were released. Seven of them come from the former EMBRAPA/CIRAD project, and six from the CIAT/CIRAD project, two are from CIRAD, and one is fromEMBRAPA-CNPAF. In 2002, the line CIAT, CT 11251-7-2-M-M-BR1 was released as TALENTO. A new CIAT/CIRAD promising line, CT13226-11-1-M-BR1, is to be released next year.

Colombia. During 2002, CIAT and CORPOICA worked together to release “Línea 30” or CIRAD 409. Trials of efficiency were set-up at the LES and on-farm in the Colombian savannas. Outside of Colombia, “Línea 30” shows good adaptation in Pucallpa Peru and is in the process of impact assessment for the slash and burn ecosystem (Efrain Leguia and Douglas White; CIAT Pucallpa-Peru, personal communication).

Line Registration

CIRAD has a mechanism by which breeders of the institution can register promising genetic material. In 2001, we applied for the registration of the upland line CT 10069-27-3-1-4 developed by CIAT/CIRAD. The line is well adapted to the mid-altitude hillsides (up to 1450 masl) in inter-cropping with young coffee plantations. In 2002 it was registered as CIRAD 445 and genetic basic seed wad produced (30 individual panicle rows).

Inter-Specific Crosses

From the 338 NERICA lines introduced from WARDA, 74 were selected (selection index: 22%). From the 79 inter-specific progenies from CIAT (45 from *Oryza sativa* by *O. glaberrima*, 24 from *Oryza sativa* by *O. barthii)*, 10 were selected (selection intensity: 13%). Results of the evaluation trial of the 314 lines from Caiapo/ *Oryza glaberrima* are not yet available.

Conclusion

The conventional backcross breeding projects of CIAT and CIAT/CIRAD continue being a source for the release of new varieties. But the released lines present narrow genetic base, which needs to be broadened. Inter-specific crosses as well as population breeding through recurrent selection are new breeding methods in use by CIAT and CIAT/CIRAD to reach this objective.

Future Activities

- Renewing the upland genetic resources stored at CIAT.
- Seed multiplication of the selected lines for evaluation and selection by LAC NARS.
- Implementation of the upland VioCIAT nurseries.

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- **Rice (*Oryza sativa* L.) Composite Population Breeding Aerobic Upland Rice (*Oryza sativa* L., Japonica type) for the Savannas Ecosystem**

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

Abstract

Since 1996, following CIAT's recommendations, the CIAT/CIRAD projects have phased out *Oryza sativa* conventional crossbreeding activities and concentrate on broadening the genetic base of rice. The development and enhancement by recurrent selection of upland rice populations are the new breeding strategies to achieve the objective. Using a recessive male-sterile gene (ms), the development of rice population was eased. In Colombia, upland basic composite populations were enhanced using recurrent selection-breeding methods. At each enhancement cycle, fertile plants are selected and are the starting-point of progeny selection. In 2002, a total of 619 lines were evaluated. Advanced lines are evaluated in yield trials with CORPOICA.

Keywords: Upland rice, composite populations, genetic base, male-sterile gene, enhancement, recurrent selection, and promising lines.

Introduction

The former conventional backcross breeding projects of CIAT and CIAT/CIRAD continue being a source for the release of new varieties. In Latin America, conventional upland crossbreeding projects have permitted the release of 33 varieties in different countries. But the released lines present some narrow genetic base which need to be broadened. Population breeding through recurrent selection is a new breeding method used by CIAT and CIAT/CIRAD to reach the objective of broadening the genetic base of upland rice. Since 1996, the CIAT/CIRAD project has concentrated on the development and enhancement of upland rice gene pools (*Oryza sativa* L., Japonica type). This strategy is feasible because it uses a recessive male-sterile gene (ms) from a mutant of IR36. Basic populations were enhanced using two recurrent selection-breeding methods. From the basic germplasm and at each enhancement cycle, fertile plants are selected and these become the starting point for the generation of segregating progenies, which are then evaluated and selected by conventional pedigree method. The number of progenies developed from population breeding has steadily increased from 1997 on. In 2002, 642 lines were evaluated and selected. We shipped basic and enhanced populations, as well as segregating lines to regional Latin American NARS (Bolivia, Brazil and Nicaragua) for local evaluation and selection. The most advanced lines were evaluated in yield trials in collaboration with CORPOICA in the Llanos of Colombia.

Material and Methods

Source of germplasm

The populations PCT-4 and PCT-11 were developed in Colombia, and the population CNA 7 was developed in Brazil, these are the basic germplasm for recurrent selection breeding.

Development of Segregating and Fixed Lines

During the cycles of genetic enhancement as well as during the recombination phases, fertile plants were selected, and were the starting-point for segregating and future fixed progenies. In 2002, we evaluated and selected 642 progenies in different segregating generations (S_1 – S_9).

Yield Trials

During the cropping season 2002, 24 advanced lines and three commercial checks (Oryzica Sabana 6, Oryzica Sabana 10, and "Línea 30" -CIRAD 409-), were evaluated in preliminary yield trials with CORPOICA.

Composite Population Breeding

Population breeding by recurrent selection is very efficient for trait improvement showing low heritability. Through short selection-recombination cycles, linkage blocks can be broken down and favorable genes accumulated. This is a process of continuous improvement.

Sowing Composite Populations: Rice populations that were developed using fertile (Msms) and male-sterile plants (msms) allow natural cross-pollination. The populations were planted in individual hill plots to facilitate the identification of male-sterile plants where recombination occurs. To encourage the recombination between early and late flowering material, two to three sowing dates were made in the same plot. To avoid pollen contamination from other rice plots, each population was surrounded by a border of maize.

Recombining and Multiplying Composite Populations: Grains produced by male-sterile plants are Msms and msms (pollen produced by fertile plants is ms or Ms and female organs of male-sterile plants are ms). Harvesting the male-sterile plants represents a new cycle of recombination as well as seed multiplication of the population.

Enhancing Composite Populations by Recurrent Selection: Recurrent selection is a cyclic process involving three main steps: plant selection (selection unit), evaluation and recombination (recombination units) of the best performing selection units. Two recurrent selection methods where used: mass recurrent selection and S_2 progenies evaluation.

Selecting Fertile Plants for Line Development

The selection of S_0 fertile plants (Msms) was the starting point for segregating line development. Selecting and harvesting only fertile plants eliminated the male-sterile gene. Advanced progenies were highly fertile (MsMs), and line development follows traditional evaluation and pedigree selection. The major characteristics selected for the savanna conditions were early vigor, tolerance of soil acidity, resistance to rice blast (*Pyricularia grisea* Sacc.) and the rice plant-hopper (*Tagosodes orizicolus*), good grain quality (translucent, long-slender grain) and early maturity (total cycle about 115 days).

Yield trials

Promising lines from different breeding populations were selected during the last years. Some of them were evaluated in preliminary experimental yield trials in Colombia, at La Libertad experimental station (LES) and on-farm, in collaboration with CORPOICA Regional 8. The experimental design was of randomized blocks with 3 replications.

Results and Discussion

Population Breeding at La Libertad Experimental Station (LES)

Population enhancement was made through recurrent selection. Two different methods were used. Mass recurrent selection on both sexes (before flowering) for resistance to total rice leaf blast and Hoja Blanca Virus and selection based on S_2 lines for main agronomic traits.

Shuttle Breeding between Colombia and Bolivia

In Bolivia, 90% of the rice is grown in favorable upland conditions, using both mechanized and manual cropping by large and smallholders, respectively. Through the years of joint germplasm evaluation, we noticed the excellent behavior and adaptation of the upland germplasm developed by CIAT/CIRAD. The environmental conditions of Santa Cruz de la Sierra are very similar to those present in Colombia, although the soils in Colombia are more acidic than in Bolivia. This makes it feasible to use a shuttle-breeding strategy. Because the cropping seasons are different in Bolivia (October-March) and in Colombia (April-September), this allows two cycles every year. In 1999, after completing the first cycle of recurrent selection in Colombia, the composite populations PCT-4 and PCT 11 from CIAT/CIRAD and the population CNA-7 from EMBRAPA-Brazil where shipped to the "Centro de Investigación Agrícola Tropical de Santa Cruz", (CIAT SZ) Bolivia for evaluation and selection.

Shuttle Breeding Steps

Bolivia CIAT SZ

-1999. Introduction of composite populations

-Cropping season 1999-2000: Evaluation and characterization of the composite populations. Selection of fertile S_0 plants in each germplasm.

-Cropping season 2000-2001: Evaluation and selection of the S_1 progenies and shipping to Colombia of the seeds of the selected progenies.

Colombia CIAT Palmira

-2001: Recombination of the selected progenies, obtaining the enhanced populations with a second cycle of recurrent selection. Seeds of the enhanced populations were shipped back to Bolivia. During the recombination cycle fertile plants were selected for evaluation and selection in Colombia.

Bolivia CIAT SZ

Cropping season 2001-2002: Sowing the enhanced populations and starting a new cycle of recurrent selection in Bolivia.

Colombia CIAT (LES)

Cropping season 2002: Sowing the enhanced populations and starting a new cycle of recurrent selection in Colombia.

Line Development

During the cropping season 2002, at LES, 642 lines were evaluated and 112 selected (selection index: 19%). In the early segregating progenies (S_1 – S_4) 145 individual plants were selected in 56 progenies. Sixty-five selected advanced progenies (S_5 – S_9) were harvested in bulk. The most advanced selected lines will be shipped to the countries were the CIAT/CIRAD project have cooperators (Argentina, Bolivia, Brazil, Colombia, Cuba, Nicaragua, Honduras and Venezuela) for local evaluation and selection. This will be done through the new germplasm nursery networks, (VioCIAT).

Yield trials

During the cropping season 2002, in collaboration with CORPOICA, yield trials of 24 advanced lines and 3 commercial checks (Oryzica Sabana 6, Oryzica Sabana 10, and “Línea 30”-CIRAD 409-) were set-up. The trials were conducted in 5 locations including 2 at LES and 3 on-farm in savanna conditions. Results for the 2002 cropping season are not yet available, and we are reporting the results of the two previous years (Table 4).

The combined analysis of the years 2000 and 2001 at LES (Table 4.) shows that grain yields are between 2000 and 3488 kg/ha. The checks “Línea 30”-CIRAD 409-, Oryzica Sabana 6 y Oryzica Sabana 10 yielded 2931, 2633 and 2000 kg/ha respectively. The line PCT-4\SA\1\1>975-M-2-M-3 showed excellent behavior, yielding 19, 32 and 74% more than “Línea 30” -CIRAD 409-, Oryzica Sabana 6 and Oryzica Sabana 10, respectively. Furthermore, it is as early as the earliest check “Línea 30”-CIRAD 409. This confirms that it is possible to breakdown the negative correlation between earliness and grain yield. From another point of view, the line does not present annual yield fluctuations, as it is the case for the checks, suggesting a more stable yield potential.

Twelve lines show similar yielding potential as the best check “Linea 30”-CIRAD 409-. These lines represent a diversified option for upland rice in the Colombian savannas and could contribute diversified the genetic material for the producers.

Table 4. Promising Line Selected from the Upland Rice Composite Population PCT-4. LES, Villavicencio-Meta, Colombia 2000 and 2001

Promising line and Checks	Year and Yield (Kg/ha)			Flowering (days)
	2000	2001	Average	
PCT-4\SA\1\1* >975-M-2-M-3	3644	3333	3488	71
Línea 30 (CIRAD 409)	2332	3531	2931	71
Oryzica Sabana 6	2140	3126	2633	83
Oryzica Sabana 10	1240	2770	2000	89

* PCT-4\SA\1\1: Nomenclature for the population PCT-4. One cycle of recurrent selection (selection cycle for acid soil –SA--, followed bay a recombination cycle.Regional Networking Activity

One of the activities of the CIAT/CIRAD project relates to the training of rice breeders in the use of recurrent selection and monitoring the progress made by regional LAC NARS, and were part of the Shuttle Breeding between Colombia and Bolivia. Other activities were the National workshop in Cuba and the International workshop in Bolivia.

National Workshop in Cuba

The "Instituto de Investigaciones de Arroz de Cuba" (IIA) and the CIAT/CIRAD jointly organized a national workshop in June 2001 in Sancti-Spiritus, Cuba. The main objective was to inform Cuban scientists about the rice recurrent selection activities in progress in Cuba, and to present the regional activities of the CIAT/CIRAD project.

International Workshop in Bolivia

CIAT SZ, DISAPA, CIAT/CIRAD and EMBRAPA Rice and Beans Center jointly organized the second international upland workshop in Santa Cruz de la Sierra-Bolivia (March 4-9, 2002).

The main objectives of the workshop were to:

1. Promote the integration of the upland rice breeders of the region.
2. Share experiences in the management of breeding populations and the development of fixed lines.
3. Select under field conditions segregating and fixed lines to be introduced in each respective country (much better than only shipping-out lines).
4. A report on the outputs and results of the Bolivian upland rice project
5. To coordinate the process to gather information on the behavior and adaptation of the selected lines in Bolivia.

Upland rice nurseries were set-up in the CIAT SZ experimental station of Saavedra and in smallholder's farms in San Juan de Yapacani. These were used to identify and discuss selection criteria, both for the mechanized and manual upland rice ecosystem of Bolivia. Fourteen breeders representing Bolivia, Brazil, Colombia y Cuba attended the workshop;

and made technical presentations. The group decided to have the next workshop in 2004 in Cuba.

Conclusions

Three upland rice composite populations were in the enhancement process by recurrent selection and represent a reservoir for line development.

Promising lines have been selected for the mechanized upland rice ecosystem in Colombia and Bolivia and the manual ecosystem practiced by small-framers in Bolivia.

In Colombia, after two years of evaluation, the line PCT-4\SA\1\1>975-M-2-M-3 shows promising results with higher grain yield than the best commercial check and the same earliness. Furthermore it presents a more stable yield potential than the checks.

The on-going networking activities are uniting a group of upland rice breeders in Latin America and the Caribbean.

Future Activities

- To continue the enhancement of upland rice composite populations.
- To confirm in Colombia the behavior of the best selected lines through collaboration with CORPOICA regional 8.
- To release new upland rice lines in the region.
- To follow-up regional network activities.
- To continue the shipping of segregating and fixed lines as well as enhanced composite populations to the cooperators of the CIAT/CIRAD project and also to Dr. Gilles Trouche responsible for the new collaborative CIAT/CIRAD project on participatory rice breeding in Central America.
- Implementation of the upland VioCIAT nurseries network.

• Irrigated Lowland Rice (*Oryza sativa* L., *Indica* and *Japonica* Type)

Châtel M., Ospina Y., Martínez C.P., Guzmán D.

Abstract

The upland rice project seeks to broaden the genetic base of lowland rice through recurrent selection. Basic populations were developed at CIAT and distributed to LAC NARS for evaluation. Site-specific *indica* and *japonica* populations for the tropics and temperate areas, respectively, were developed with the NARS, and were the starting-point of rice recurrent selection activities.

Keywords: Lowland rice, rice populations, genetic base, male-sterile gene, enhancement, site-specific populations, and recurrent selection.

Introduction

Population Breeding for Lowland irrigated rice is done in close collaboration with LAC NARS and CIRAD in France. The breeding population project started by introducing to Colombia different gene pools and populations previously developed in Brazil by CIRAD/EMBRAPA–CNPAF and CIRAD. Populations were created by CIAT/CIRAD to target the different rice ecosystems presents in LAC (Tropics, sub-tropics and temperate climate). Since 1996, this basic germplasm has continued to be dispatched to our regional cooperators for local evaluation and characterization. The best adapted composite populations were the starting point of population breeding activities in different countries as well as the development of site-specific ones.

Following networking activities, the CIAT/CIRAD project and Fundación DANAC-Venezuela organized, in October 2001, the First International Workshop on rice recurrent selection for irrigated rice. This year we started the organization of the III International Conference in rice population breeding to be held in May 2003 in Venezuela.

Activities presented were conducted at CIAT Palmira experimental station (PES) and with our partners in their countries. Additionally population breeding activities directly conducted by NARS and monitored by the CIAT/CIRAD project are to be presented in a book to be published by CIAT/CIRAD (Colombia), EMBRAPA (Brazil), Fundación DANAC (Venezuela) and FAO, at the beginning of 2003 for the III International Conference

Materials and Methods

Basic Populations: In late 1996, the CIAT/CIRAD composite populations PCT-6, PCT-7, PCT-8 (*Indica* type) and GPIRAT-10 (*Japonica* type) were shipped to LAC NARS for local evaluation and characterization.

Site-Specific Populations: Following the evaluation and characterization of the introduced germplasm, LAC breeders, in coordination with CIAT/CIRAD, started the creation of site-specific populations.

Development of Segregating and Fixed Lines: From the introduced and site-specific populations, LAC breeders started selecting S_0 fertile plants that are the starting point for line development.

Site-Specific Populations: Site-specific populations were created by the introduction of locally selected germplasm identified by NARS breeders into an introduced CIAT/CIRAD populations that had a diverse genetic background and were segregating for a male-sterile recessive gene.

Development of Segregating and Fixed Lines: Starting from selected S_0 fertile plants, the progenies were evaluated and selected by conventional pedigree method. Segregating progenies were evaluated and only fertile plants were selected to eliminate the male-sterile gene. The best-advanced fertile lines were tested in nurseries and then best promising lines in regional trials.

Results and Discussion

The results presented here after correspond to the activities conducted at CIAT-PES in collaboration with NARS breeders.

New Site-Specific Populations

Argentina (Sub-Tropical Rice Ecosystem): During 2000 and 2001, the site-specific population PARG-3 was synthesized by introducing in the CIAT/CIRAD population PCT-8 of six (6) parents identified and selected by the Argentinean breeders. The population was shipped to Argentina for evaluation and selection during the cropping season 2001/2002.

Chile (Temperate Climate Rice Ecosystem): The site-specific population PQUI-2 is being developed in Chile for cold temperature tolerance, yielding potential and grain quality. The source of adapted genetic and male-sterility background comes from the population PQUI-1 previously developed and selected in the country. Twelve parents showing good adaptation to cold tolerance and being genetically divergent from the genetic constitution of PQUI-1 represent the new variability incorporated to PQUI-1. They come from the CIRAD cold tolerance project in Madagascar (5 parents), the Chilean breeding project (6 parents) and IRRI (1 parent). The crosses between each one of the new parents and male-sterile plants of the population PQUI-1 were made in Chile, as well as the evaluation of the F_1 generation. Because Chile has only one cropping season, seeds of the F_2 generation of the PQUI-2 population were harvested in bulk and part of it was shipped to CIAT for recombination in 2001. The PQUI-2 recombined population will be shipped back to Chile for sowing during the cropping season 2002/2003.

Uruguay (Sub-Tropical/Temperate Climate Rice Ecosystem): Three site-specific populations were developed at CIAT-PES: PURG-1 with round/medium grain type for rice exportations to the Asian market, PURG-2 with long-slender and high grain quality for the rice exportation to the Middle East market and PURG-3 for long term population enhancement. During the development of the populations PURG-1 y PURG-2, 40 fertile plants were selected for their evaluation to cold tolerance by FLAR.

France and Chile (Temperate Climate Rice Ecosystems): In countries where rice productions and consumption are relatively low, there are specialized market niches, and it is important to diversify of different rice types to attract potential new consumers. Market niches are also very attractive to the producers and the rice industry who benefits from the high aggregate value of the specialized rice. In France aromatic rice are popular, and in Chile, the industry and some consumers are looking for new rice types. Rice aroma is a very complex trait to deal with by conventional breeding because it involves a great number of major and minor genes. Population breeding using recurrent selection is a method adapted to deal with complex traits. It was decided to develop a new site-specific population to be enhanced both in France and Chile. As rice cropping season are inverted between France and Chile, shuttle-breeding will speed up the development process. Twenty-six aromatic and non-aromatic parents were selected by CIRAD-France, and the population PQUI-1 from Chile was selected as good genetic with a male-sterile background that is well adapted to the rice temperate climate ecosystem. Individual crosses between each new source of variability and male-sterile plants of the population PQUI-1 were made in 2001 at CIAT-PES. The F_1 generation of each one of the 26 crosses was sown and the F_2 seeds harvested separately. Part of the F_2 seeds of each individual cross was shipped to CIRAD-France for evaluation during the cropping season 2002 (April to September). At CIAT-PES, the other part of each individual F_2 seed was bulked in equal proportion to obtain the basic population named PACQ-1. The basic population was sown to obtain the first cycle of recombination. From the basic recombined population the seed will split in three parts. One part to be stored in the CIAT, another part is to be shipped to Chile for its evaluation during the cropping season 2002/2003 (October to March), and the third part is to be shipped to CIRAD-France for evaluation during the cropping season 2003.

Colombia: Taking in account that a major objective of the CIAT rice project is to broaden the genetic base of rice through advanced breeding methods. To further this aim, it was decided to use recurrent selection to develop populations that included inter-specific crosses. Two new populations were set-up by introducing seven advanced inter-specific advanced lines (six from the cross *Oryza rufipogon*/Bg90-2, and one from the cross *Oryza barthii*/Lemont) into the Venezuelan tropical site-specific population PFD-1 and the Argentinean sub-tropical site-specific population PARG-3. The new populations were named PCTFD-20 and PCTARG-19, respectively. In relation to the already existing populations managed by CIAT/CIRAD and LAC NARS, the new germplasm integrates additional genetic variability. The two new populations will be enhanced first in Colombia before being shipped to LAC NARS.

Development of Segregating and Fixed Lines

Each one of the LAC rice breeders involved in composite population breeding is very conscious that the objective of their work is to produce commercial varieties. The most advanced rice population breeding projects are in Brazil, Argentina, Chile, Cuba and Venezuela. Each one has evaluated and selected line gardens and then conducted regional trials growing the most promising lines.

Brazil: The collaborative project between EMBRAPA Arroz e Feijão (former CNPAF) and CIRAD-CA (former IRAT), developed in 1989 the lowland rice genetic pool CNA-IRAT 4. Breeding this genetic pool by two cycles of recurrent selection lead to the selection and evaluation of lines extracted from the enhanced germplasm by different rice research institutions in Brazil (National Network organized by EMBRAPA Rice and Beans). This work resulted in 2002 in the release of the line “Tio-Taka” by the “Empresa de Pesquisa Agropecuária e Extensão Rural” (EPAGRI), of the State of Santa Catarina.

- **This is The First ever Rice Line Released in Latin America Coming from the Enhancement of a Gene Pool by Recurrent Selection**

Other important results are coming-out in different Latin American countries. For example, in Chile during the cropping season 2001/2002, there were 10 line gardens representing a total of 886 segregating and advanced lines from the population PQUI-1 in different cycles of enhancement. Seventeen promising lines were evaluated with three commercial checks in a yield trial. The results of the trial show that five of the lines yielded more that the checks of Oro and Diamante, and three are yielding slightly more than the best commercial variety Brillante.

Regional Networking Activity

One of the activities of the CIAT/CIRAD project relates to the training of rice breeders in the use of recurrent selection and monitoring the progress made by regional LAC NARS.

Conclusion

In different LAC countries, and for different rice ecosystems, rice composite population breeding is a reality and NARS breeders have a great compromise in their collaborative work with the CIAT/CIRAD project.

Two of the breeding strategies of the CIAT rice project were merged by the creation of new broad genetic base populations using inter-specific advanced lines.
Segregating and advanced lines are being evaluated and selected by each LAC rice program, and promising lines are coming-out

Future Activities

- To finalize the creation of the new site-specific populations
- To follow-up supporting rice LAC breeders
- To ensure the development and selection of promising breeding lines and their release as commercial varieties by LAC rice breeders

Producing Upland Rice with Young Coffee Plantations in the Colombian Hillsides

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Abstract

Production of upland rice in the hillsides of the Colombian coffee zone is an option for both food security and land use management of young coffee plantation. To evaluate the effect of two cycles of rice cultivation on young coffee plantations, an experiment was conducted at the experimental stations La Catalina, Pereira – Risaralda, and Naranjal, Chinchiná - Caldas. The results of inter-cropping upland rice with coffee indicate that coffee did not affect rice production. Average rice-paddy production varied between 3760 and 4736

Kg/ha, depending of the year and site of evaluation. At Naranjal, rice did not affect the subsequent coffee productions, but at La Catalina, it varies according to the spatial arrangement of the coffee. The highest coffee production (Naranjal: 8253 and La Catalina: 7566 kg/ha of dry grain) was reached by using a rice-sowing density of 60 Kg/ha, distributed in 3 rows with spatial arrangement of coffee of 1,00x1,00 m.

Keywords: Upland rice, coffee, inter-cropping, hillsides.

Introduction

In the Colombian coffee region, the production of staple foods with the exception of plantains, are not enough to reach self-sufficiency. Agronomic solutions were proposed to upgrade the livelihood of the coffee producers by inter-cropping food crops with young coffee plantations while waiting for the first coffee harvest. It is also a way to better manage land use in the hillsides. The "Centro Nacional de Investigación de Café" (CENICAFÉ) conducted experimental inter-cropping of tomatoes, beans, maize, and rice with in young coffee plantings. Upland rice was for the first time introduced in the Colombian hillsides in 1993. Experimental results of the adaptation of upland rice led to the identification and selection of one upland savanna rice line adapted to the mid-altitude hillsides (up to 1425 m). It was decided to study the inter-cropping of upland rice and coffee.

Material and Methods

Site: The experiment was set-up in two experimental stations of CENICAFE: La Catalina, Pereira – Risaralda, at an altitude of 1310 m, and located at 4° 45´ North and 75° 44´ West, and Naranjal, Chinchiná, Caldas, at an altitude of 1425 m and located 4° 58¨ North and 75° 39¨ West.

Germplasm: Upland lines developed by the CIAT/CIRAD rice project for the Colombian savanna ecosystem were selected for earliness, because of vegetative and reproductive cycle elongation with cold temperature in altitude. They were evaluated in collaboration with CENICAFE during 1993 and 1994 (Moreno et al, 1996). The upland savanna rice line CT10069-27-3-1-4 (CIRAD 445) was selected for presenting good yielding ability and low spicklet sterility which is indirect indicator of cold tolerance. The coffee variety used was Colombia, for its high yielding potential and resistance to the coffee rust (*Hemileia vastatrix* Berk. y Br.).

Experimental Design: The experiment was an augmented factorial design 3x3+1, with ten treatments and three sowing distances between coffee plants (1,00x1,00 m; 1,42x1,42 m and 2,00x1,00 m), three rice sowing densities (60, 80 and 100 kg of seed /ha). For maintaining the same population (10,000 plants/ha) of coffee plants, only one plant was grown when spacing was 1x1m, and two plants for the spacing 1.42x1.42 and 2.00x1.00 m. The design was of complete blocks with five replications. Coffee spacing represented the principal plot and rice densities were the sub-plots. The evaluation was on rice paddy-grain production/ha and coffee fruit production later on transformed to dry coffee grain.

Sowing Upland Rice: Rice was direct seeded by drilling seeds, in 3, 4 and 6 rows depending of the spatial arrangement of the coffee plants, 1,00x1,00 m, 1,42x1,42 m and 2,00x1,00 m respectively.

Crop Management: The management of the two crops was made according to their respective necessities and agronomic practices. The fertilization both for coffee and rice was made according to soil analyze and crop requirements. The first rice sowing was with minimum tillage just after the sowing of the coffee plants. The fertilization was 25 kg/ha of P at sowing and 80 kg/ha of N (a third at sowing, a third at tillering and the last third at flowering). Rice harvesting was manual, cutting the panicles and leaving the straws on field. Six month old coffee plants were sowed and the fertilization was 150, 200, 250 and 300 kg/ha of urea at 1, 5, 9 and 13 months respectively. After 18 month and at each subsequent 6 months, 700 kg/ha of the formula 17-6-18-2 were applied.

Results and Discussion

Upland-Hillside Rice Production

The results confirmed that the upland-savanna rice line CT-10069-27-3-1-4 (CIRAD 445) has good adaptation to the mid-altitude hillsides of the Colombian Coffee region, and that it was feasible to grow selected upland-savanna rice germplasm up to an altitude of 1450m.

La Catalina (1310 m): Data analysis of the two years of experiment did not show any effect of coffee plantation on rice production. Furthermore rice-sowing density did not effect the rice production. The good vigor of the rice plants permitted good competition with coffee plants and sowing density of 60 Kg/ha was enough to reach good yields. These results show that it is possible to inter-crop rice with young coffee plantations during two cropping seasons. The accumulated paddy-rice production reached about 8,000 Kg /ha. By manual harvesting the rice panicles, and leaving the vegetal material on the ground, it helps prevent soil erosion and weed infestation.

Naranjal (1425 m): In each spatial coffee arrangement, the rice sowing densities did not affect rice-paddy production. Nevertheless, they affected rice-paddy production at each rice-cropping season. During the cropping seasons 1996 and 1997, the best rice production, 4250 Kg/ha and 5712 Kg/ha, were with coffee sowed at 1,42x1,42m and 1,00x1,00m respectively. Independent of coffee spatial arrangements and rice sowing densities and number of rows, rice production was 26% higher during the cropping season 1997 than in 1996. The residual fertilization of the year 1996 is perhaps responsible of this difference in rice yield. In conclusion, and as well as in La Catalina, rice density of 60 kg/ha is enough, but the number of rice rows has to be no more than 3 to reach the best production during the second cropping season, with coffee spacing of 1,00mx1,00m

Coffee Production

In Colombia, Mestre and Salazar (1995) showed that coffee production, grown in monoculture is not affected by spatial arrangements, by the number of plants or the sowing site, when a density of 10,000 plants/ha is used (Table 6). In the experiments conducted in both sites, when inter-cropping coffee and upland-hillside rice, the coffee production depended on the spatial arrangement.

La Catalina: The highest coffee production (average of 3 harvests: 7566 Kg/ha of dry grain) was with 1,00x1,00 m and one coffee plant per site. Reductions of 30 and 25% of the production is observed with the spatial arrangements 1,42x1,42 m and 2,00x2,00 m and two plants per site, respectively. This can be explained because of the presence of more rice rows and subsequent competition with coffee. In some cases rice completely covered and shadowed the coffee plants. But this was manageable by reducing the number or rice rows what was not a problem for rice production, because the yields were independent of the sowing density or number of rows at the levels used in these trails. Coffee production was higher in 1998 than during 1997 and 1999. This is due to the intrinsic behavior of coffee production, which is biennial with an alternate high and low production rate.

Naranjal: As was the case in La Catalina, the best coffee yield (average of 2 harvests: 8253 Kg/ha of dry grain) were scored in each one of the harvest with a spatial arrangement of 1,00x1,00 m and one plant per site. Reduction in coffee production observed with the coffee arrangements1,42x1,42 m and 2,00x2,00 m and two plants by site are explained by the same considerations than in La Catalina, and the solution is the same one. Coffee production was higher in 1999 and is because of the biannual production cycle of coffee.

Conclusions

Upland rice was first introduced to the Colombian coffee hillsides region in 1993. Different upland savannas rice lines from the CIAT/CIRAD project were evaluated and the line CT-10069-27-3-1-4 (CIRAD 445) was selected for its good adaptation (yield potential and tolerance to cold temperature) to mid-altitude conditions.

The results of the experiments of rice inter-cropping with coffee indicate that the average rice production is between 3760 and 4736 Kg/ha of paddy-rice, depending of the year and site of evaluation. The highest production can be reached by using a sowing density of 60 Kg/ha of rice seed, distributed in 3 rows. The highest coffee production, with an average of 8253 and 7566 kg/ha of dry coffee grain, was when coffee is planted at a spatial arrangement of 1,00x1,00 m and one plant per site, in Naranjal and La Catalina respectively.

These results confirmed that when in association, the adaptation of spatial arrangements of the crops is important in order to maximize the production of each one by minimizing competition effects. Upland-hillside rice inter-cropped with young coffee trees permited an economically viable level of production of this staple food and did not affect the subsequent coffee production. In that sense it is a reasonable option for increasing food security in the Colombian hillsides, and a sustainable way of producing coffee. Furthermore, selling surplus of production can improve the livelihood of the coffee producers.

Future Activities

- To share the results with the Coffee producers community. Contact was already made with Dr. José María Astaiza, Executive Director of the Cauca Coffee Committee.
- To officially release the upland rice line CT-10069-27-3-1-4 (CIRAD 445) .

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Table 5. Upland-hillside Rice Production in Inter-Cropping with Young Coffee Plantations. La Catalina and Naranjal, 1996 and 1997 Cropping Seasons

Coffee (10.000 plants/ha)	Upland-Hillside rice		Paddy-rice production (kg/ha)					
			La Catalina			Naranjal		
	Seed density (kg/ha)	Number of row	1996	1997	Average	1996	1997	Average
1,00x1,00m (One plant by site)	60	3	4275	3866	4070	3338	5931	4634
	80	3	4541	4100	4320	3256	5469	4362
	100	3	4728	4293	4510	3342	5736	4539
1,42x1,42m (Two plants by site)	60	4	4184	3768	3976	4158	4140	4149
	80	4	4285	3855	4070	4201	4769	4485
	100	4	4216	3796	4006	4389	4626	4507
2,00x1,00m (Two plants by site)	60	6	4542	4102	4322	3659	4283	3971
	80	6	3764	3354	3559	3562	4386	3974
	100	6	3694	3269	3481	3934	4549	4241
Average			**4248**	**3822**	**4035**	**3760**	**4876**	**4318**

Table 6. Coffee Production in Inter-Cropping with Upland-Hillside Rice. La Catalina, 1997, 1998 and 1999 and Naranjal, 1999 and 2000

Coffee (10.000 plants/ha)	Upland-Hillside rice		Coffee Dry Grain Production (kg/ha)						
			La Catalina				Naranjal		
	Seed density (kg/ha)	Number of row	1997	1998	1999	Average	1999	2000	Average
1,00x1,00m (One plant by site)	60	3	4940	11231	5668	7280	10031	7823	8927
	80	3	5953	10217	6650	7606	9994	5790	7892
	100	3	5981	10686	**6770**	7812	9271	6608	7940
1,42x1,42m (Two plants by site)	60	4	1681	3497	1210	2129	5762	4099	4930
	80	4	1506	4198	1333	2346	5396	3483	4439
	100	4	1932	4154	1177	2421	5220	3287	4253
2,00x1,00m (Two plants by site)	60	6	1461	3277	1249	1996	4752	2960	3856
	80	6	1303	3076	1153	1844	6770	3274	5022
	100	6	1401	3228	1082	1904	5863	3239	4551
Average			**2906**	**5952**	**2921**	**3926**	**7266**	**4879**	**6072**

OUTPUT 1. ENHANCING GENE POOLS

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

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

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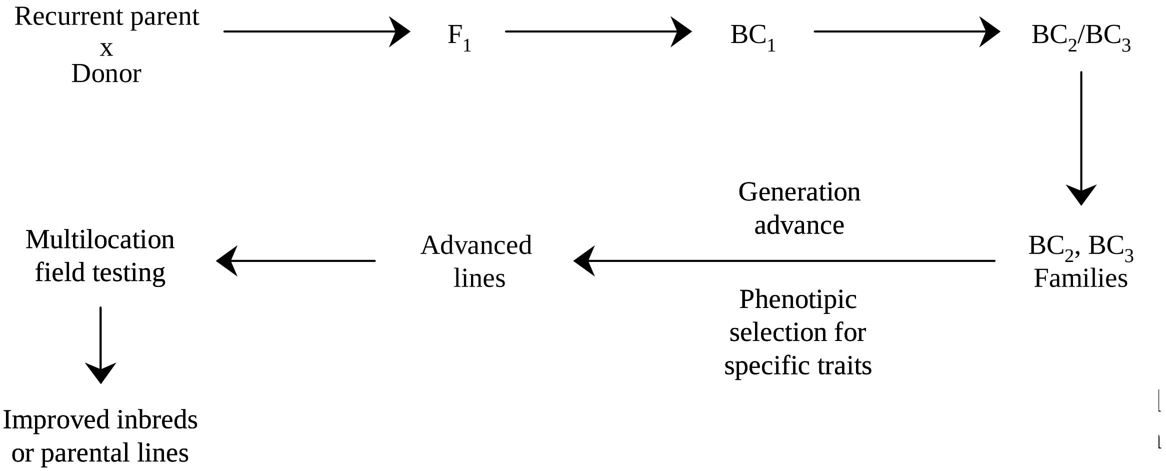
Introduction

In spite of the great impact made in rice production in Latin America (LAC) there is a need to increase it in a sustainable way. New alleles can provide genetic variability for crop enhancement. The Oryza wild species represent a potential source of new alleles for improving the yield, quality and stress resistance of cultivated rice. The purpose of this work is to provide increasing evidence that certain regions in *O. rufipogon* and *O. glaberrima* harbor genes of interest for the genetic improvement of cultivated rice.**Materials and Methods**

Breeding Scheme

Improved varieties (Bg90-2, Oryzica 3 and Caiapo) were used as the recurrent parents while *O. rufipogon* (IRGC105491), and O. *glaberrima* (IRGC103544) were used as donors in the interspecific-backcross breeding scheme shown below.



Argentina) in close collaboration with key partners from the national rice programs and the private sector. Transplanting was done in CIAT and direct seeding was done elsewhere. A completely randomized design with three replications was used and crop management was based on recommended local agronomic practices. Locally popular varieties were used as checks. Data on the main agronomic traits, including grain yield, were taken. A two-way analysis of variance was used for the analysis of grain yield, and a GEBEI package that implements appropriate clustering and ordination procedures was used in a preliminary analysis of the genetic by environment (GxE) data.

Evaluation for Tolerance to Biotic Stresses

Advanced breeding lines from the cross Oryzica 3/*O. rufipogon* were field tested in a “hot spot” for their reaction to three diseases in Tolima, Colombia, using the Standard Evaluation System for Rice and three replications. Doubled-haploid lines derived from the cross Caiapo/*O. glaberrima* were evaluated in a “hot spot” area in Meta, Colombia, for tolerance to the Rice Stripe Necrosis Virus. Both field tests were conducted in close collaboration with research assistants from FEDEARROZ (Colombian Rice Growers Association).

Selection for Grain Quality

Advanced breeding lines were evaluated for grain length and translucency in the CIAT Quality Lab.**Results**

Grain Yield

Data are presented in Figure 1 and Table 1. Statistical analysis showed no significant difference in grain yield between Bg90-2 and its progeny over location. Although none of the interspecific lines had yields greater than Bg90-2 in all of the locations, a few lines performed better than Bg90-2 in each location. A preliminary analysis of the GxE (data not shown) indicated that contrasting and distinct environments were included in these trials and that the GxE interaction was high (75%). This suggests that the performance of genotypes was dependent on the climatic/soil conditions in each location and that there was a good level of genetic variability present in this group of lines, and this explains the better performance of some progenies under specific conditions. This is very important for breeding purposes since the genetic variability hidden in this population was only observed when the progenies were exposed to a diverse set of climatic/soil conditions found in different rice growing areas. A few lines performed as well as Fedearroz 50, the highest yielding variety planted by farmers in Colombia.

Tolerance to Biotic Stresses

Some fungal diseases, particularly *Rhizoctonia solani*, *Sarocladium oryzae* and *Bipolaris oryzae*, considered of minor importance in the past are now causing yield losses in several areas in LAC. Most commercial varieties grown are susceptible. Results from field and greenhouse tests suggest that lines derived from the cross Oryzica 3/*O. rufipogon* showed a good tolerance level to these diseases (Tables 2, 3), particularly to *Rhizoctonia* sp. Besides some advanced lines from the Bg90-2/*O. rufipogon* cross (Table 4) are also showing tolerance to Rhizoctonia under greenhouse conditions. A preliminary evaluation of 20 accessions representing different wild rice species carried out at CIAT under control greenhouse conditions revealed additional potential sources of resistance. However, given the nature of Rhizoctonia one has to be careful about the significance of the data obtained so far. It has been claimed (Ou, 1984) that not known sources of resistance to Rhizoctonia are available in cultivated rice. Recent data from IRRI suggested that initial evaluations found some wild rice species resistant to Rhizoctonia but it turned out otherwise in further evaluations. There is a need to confirm our initial findings.

The fungus *Polymyxa graminis* transmits the rice stripe necrosis virus (RSNV), disease first reported in Ivory Coast in 1977; it was reported in Colombia in 1991 and now is found in Panama and Brazil. All commercial varieties are susceptible to RSNV; however, high level of tolerance was found in *O. glaberrima*. Our results show that tolerance to RSNV has been successfully transferred to improved varieties such as Caiapo and Bg90-2 through a backcrossing program (Tables 5 and 6).

Grain Quality

Development of high yielding varieties with tolerance to biotic stresses and excellent grain quality is the main breeding objective of national rice programs in LAC. Both parents, Bg90-2 and *O. rufipogon*, have poor grain quality. However, positive transgressive segregation for superior grain quality was observed in the BC_2F_2 generation which led to the selection of advanced lines with long and slender, translucent grains (Figure 2).

Evaluation and Selection of Segregating Populations

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

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

Nearly 200 crosses were made this year with emphasis on the recombination of positive traits exhibited by breeding lines derived from different interpecific crosses. Besides, 6287 breeding lines were evaluated during the first semester in Santa Rosa, under favorable upland conditions. Data are shown in Table 7.

Crosses between IRRI new plant type and elite lines from our gene pool have produced F_5-F_6 lines with thick and strong stems, long panicle, intermediate tillering and stay-green leaves. Although most of these lines have bold-grain type, they have a good level of resistance to several diseases.

Crosses between breeding lines derived from interspecific crosses and improved varieties or elite lines produced populations with excellent plant vigor and stems, good plant type, long and slender grains but high sterility. Selection for panicle fertility and tolerance to diseases were the main selection criteria.

Most of the lines from the Bg90-2/ *O. rufipogon* backcrosses showed high susceptibility to leaf and neck blast; however, some lines combined intermediate to good level of resistance to blast, with good plant type, strong stems, and high yield potential. These lines were selected for further evaluation in collaboration with FEDEARROZ under farmers field conditions in 2003.

Panicle and tip sterility continued to be a problem in progenies derived from the Bg90-2/ *O. glaberrima* backcrosses. Nevertheless, a good number of lines with high fertility, excellent plant vigor, sturdy stems and good yield potential were identified and selected for further evaluation to the rice stripe necrosis virus in collaboration with FEDEARROZ.

Progenies from the Lemont/*O. barthii* cross are characterized by earliness, good tolerance to foliar diseases, stay-green foliage, long/slender grain, excellent grain quality, long panicles, and high fertility. However, this material is very susceptible to Tagosodes and rice hoja blanca virus. Top-crosses were made to incorporate resistance to these pests and were run through anther culture to speed up the breeding cycle.

Finally, 248 advanced lines from the CIAT-Peru project were harvested in bulk for seed increase in Palmira. These lines are candidates for the breeding nurseries to be distributed to national rice programs in 2003. These lines are combining high yield potential, tolerance to several diseases, good early vigor, and excellent grain quality.

Discussion

It has been shown (Xiao et al., 1998; Moncada et al., 2001) that the Oryza wild species represent a potential source of new alleles for improving the yield, quality and stress resistance of cultivated rice. Our results from evaluations under greenhouse and farmer's fields confirm that *O. rufipogon* and *O. glaberrima* possess alleles with positive effects on yield, stress resistance and grain quality.

Conventional breeding methods, mainly the backcross and pedigree methods have been used in the development of improved germplasm through the utilization of wild rice species. Embryo and anther culture helped to overcome hybrid sterility. Phenotypic selection under heavy disease pressure has been used in the identification of promising breeding lines. High genetic variability for most traits of agronomic interest (early vigor, sturdy stems, yield potential, resistance to several diseases, earliness, grain quality, etc) has been observed in these interspecific crosses in our plots in Palmira and Santa Rosa. Through a collaborative approach with key partners, from both the public and private sectors, these populations have been evaluated in other rice growing regions in Colombia, Venezuela, Argentina, and Uruguay. The main feature is that our collaborators are finding good breeding material in these populations for use in their own breeding programs. This fact indicates that our main objective to broaden the genetic base of rice in LAC through the utilization of wild rice species is being met.

More recently Brar et al. (2002) reported that genes for resistance to brown planthoppers, bacterial blight, blast, tungro virus, tolerance for acidic conditions, and cytoplasmic male sterility have been transferred from wild species into cultivated rice. Evenmore, breeding lines derived from crosses of *O. sativa*/*O. officinalis* and *O. sativa/O. rufipogon* have been released as varieties in two countries. Line IR 73385-1-4-3-2-1-6 derived from IR 64/*O. rufipogon* resistant to tungro virus disease was recommended for pre-release in the Philippines. Another variety AS 996 (IR 73678-6-9-B) from the cross IR 64/*O. rufipogon* was released for cultivation in the Mekong Delta, Vietnam; it is a short-duration variety with good plant type suitable for moderately acid sulfate soils.

All together these results further underline the importance of the wild relatives of rice for the improvement of cultivated rice, as was indicated earlier by Tanksley and McCouch (1997). Not only in rice but also in other crop species such as tomato (Bernacchi et al. 1998) and maize (Goodman, 2002; Whitt et al. 2002) new exotic germplasm can have a substantial effect on productivity.

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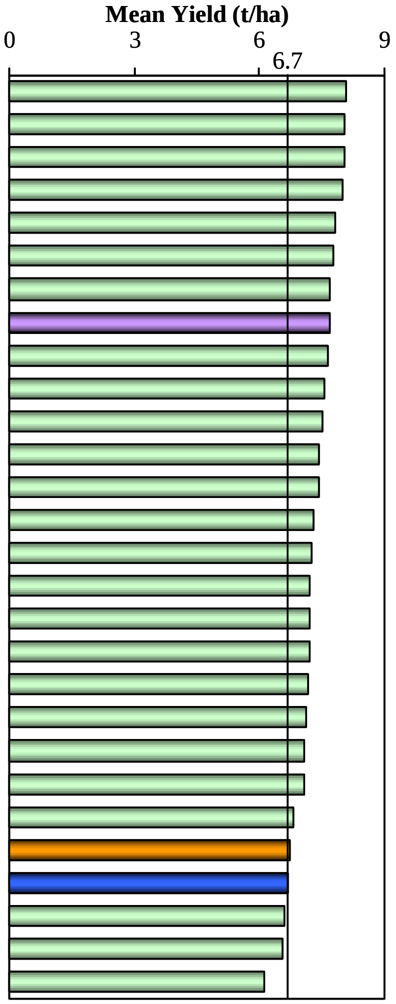
Table 1. Average Grain Yield (t/ha) and Standard Deviation (parenthesis) of Breeding Lines derived from Bg90-2/*O. rufipogon* in Farmers' Fields in Eight Locations, 2002

Nº	Pedigree	Location								Mean Yield
		Aceituno	Concepción	Montería	Saldaña	Jamundí	Villavicencio	CIAT	Armero	
01	CT13941-11-M-25-1-M-M	11.192	10.193	11.180	6.534	7.470	6.542	5.903	4.825	7.980
		(0.182)	(1.169)	(1.009)	(0.987)	(0.697)	(1.170)	(0.727)	(1.634)	(2.548)
02	CT13941-11-M-25-4-M-M	10.552	9.315	9.819	7.100	7.796	5.625	5.153	5.121	7.560
		(0.534)	(1.944)	(2.359)	(0.404)	(0.430)	(1.987)	(0.513)	(2.295)	(2.446)
03	CT13941-11-M-25-5-M-M	11.523	10.148	10.997	7.534	6.830	6.597	5.954	5.132	8.089
		(1.065)	(2.486)	(2.227)	(0.468)	(1.590)	(1.028)	(0.281)	(0.964)	(2.637)
04	CT13941-27-M-4-1-M-M	--	7.855	--	6.241	6.158	--	--	4.264	6.130
		--	(0.908)	--	(0.725)	(0.443)	--	--	(1.269)	(1.529)
05	CT13941-27-M-5-4-M-M	--	8.861	--	7.013	7.209	4.646	--	5.051	6.556
		--	(1.828)	--	(1.315)	(0.647)	(0.526)	--	(0.783)	(1.858)
06	CT13941-27-M-15-3-M-M	--	9.984	--	8.162	8.688	5.028	--	3.971	7.167
		--	(2.246)	--	(0.260)	(1.518)	(1.491)	--	(0.867)	(2.658)
07	CT13941-27-M-19-1-M-M	10.292	11.172	9.274	5.923	6.891	4.917	4.895	4.191	7.194
		(0.230)	(0.768)	(2.748)	(0.970)	(0.017)	(0.712)	(0.302)	(1.528)	(2.774)
08	CT13946-26-M-5-3-M-M	11.693	12.017	9.939	7.898	5.326	6.069	5.457	4.949	8.032
		(0.864)	(0.695)	(0.501)	(0.748)	(0.023)	(0.584)	(0.634)	(1.198)	(2.875)
09	CT13946-26-M-5-6-M-M	11.385	11.269	10.498	6.422	7.593	5.444	4.382	4.527	7.690
		(0.697)	(1.019)	(1.684)	(0.589)	(0.428)	(2.803)	(0.376)	(0.893)	(3.045)
10	CT13956-29-M-14-1-M-M	10.922	11.534	10.409	5.772	6.977	5.972	5.480	4.196	7.658
		(0.608)	(1.533)	(1.731)	(0.796)	(1.104)	(1.387)	(0.419)	(1.142)	(2.893)
11	CT13956-29-M-25-7-M-M	10.695	9.914	10.348	5.928	4.360	5.514	4.623	3.314	6.837
		(0.357)	(1.581)	(0.335)	(2.621)	(0.473)	(1.146)	(0.281)	(0.801)	(3.035)
12	CT13958-12-M-1-7-M-M	12.199	12.506	9.567	7.959	4.600	5.875	4.190	5.281	7.772
		(0.593)	(1.379)	(0.982)	(0.817)	(0.192)	(0.897)	(0.575)	(1.803)	(3.300)
13	CT13958-13-M-17-5-M-M	11.285	10.211	11.923	7.660	7.066	6.069	4.982	5.041	8.029
		(1.044)	(1.665)	(1.601)	(0.287)	(0.239)	(1.315)	(0.275)	(2.191)	(2.853)
14	CT13958-13-M-2-1-M-M	11.164	10.737	9.845	6.627	4.345	4.653	5.485	4.623	7.308
		(1.064)	(0.805)	(1.442)	(0.682)	(0.021)	(0.842)	(0.400)	(1.135)	(2.891)
15	CT13958-13-M-2-3-M-M	11.366	10.647	10.911	6.408	6.789	4.410	5.808	3.943	7.535
		(0.922)	(0.775)	(2.491)	(0.427)	(0.487)	(0.886)	(0.213)	(1.564)	(3.039)
16	CT13958-13-M-2-4-M-M	11.345	10.321	9.660	6.247	6.017	4.056	5.552	3.656	7.107
		(0.562)	(2.045)	(0.468)	(1.863)	(0.413)	(2.069)	(0.453)	(1.057)	(3.015)
17	CT13958-13-M-7-5-M-M	11.944	8.834	10.037	7.733	4.923	5.139	5.261	4.782	7.436
		(0.7)	(1.168)	(0.830)	(0.549)	(0.386)	(0.524)	(0.557)	(2.555)	(2.790)
18	CT13958-13-M-26-4-M-M	11.328	--	9.735	6.539	5.333	4.896	4.879	3.496	6.601
		(0.615)	--	(2.208)	(0.616)	(0.842)	(1.026)	(0.343)	(1.020)	(2.872)
19	CT13958-13-M-26-5-M-M	11.504	9.360	8.904	6.794	6.210	4.403	5.457	4.917	7.099
		(0.631)	(0.757)	(0.683)	(0.285)	(1.117)	(0.992)	(0.179)	(0.661)	(2.458)
20	CT13958-13-M-33-1-M-M	11.955	9.490	8.816	6.269	5.348	5.278	4.938	5.422	7.189
		(0.575)	(1.886)	(0.852)	(0.489)	(0.507)	(0.318)	(0.367)	(1.147)	(2.583)
21	CT13956-29-M-29-2-M-M	11.570	12.609	9.500	7.256	5.925	5.521	5.587	3.871	7.808
		(1.149)	(1.213)	(0.724)	(0.802)	(1.294)	(1.263)	(0.475)	(1.247)	(3.173)
22	CT13956-29-M-8-3-M-M	10.962	8.850	10.198	7.352	5.326	5.389	3.907	5.309	7.256
		(1.324)	(1.694)	(0.721)	(1.733)	(.)	(1.219)	(0.815)	(0.362)	(2.734)
23	CT13959-3-M-10-4-M-M	11.344	11.592	9.098	7.252	5.533	5.028	3.886	5.649	7.423
		(0.253)	(0.893)	(0.484)	(0.742)	(0.294)	(0.667)	(0.405)	(2.709)	(2.956)
24	CT13959-3-M-10-5-M-M	12.200	--	9.358	7.988	5.326	5.035	5.040	4.657	7.086
		(1.198)	--	(1.674)	(0.695)	(0.376)	(0.694)	(1.021)	(2.165)	(2.921)
25	CT13976-7-M-14-1-M-M	11.128	10.799	8.754	7.595	4.680	4.479	4.807	4.584	7.209
		(0.781)	(0.984)	(2.117)	(0.449)	(0.226)	(0.276)	(0.583)	(2.358)	(2.939)
26	Bg90-2	10.757	--	9.435	7.837	5.686	4.340	4.902	3.785	6.677
		(1.201)	--	(0.383)	(0.178)	(0.820)	(0.574)	(1.063)	(0.599)	(2.635)
27	Fedearroz50	10.466	--	9.953	7.811	8.621	5.000	5.372	6.469	7.670
		(0.438)	--	(0.093)	(0.387)	(2.084)	(0.722)	(0.359)	(1.109)	(2.197)
99	Local checks	10.235	--	8.212	6.144	6.504	5.042	2.927	4.322	6.733
		(0.085)	(0.348)	(1.303)	(0.602)	(1.173)	(1.081)	(0.816)	(0.425)	(2.386)

Figure 1. Performance[1/] of breeding lines derived from Bg90-2/*O. rufipogon* in farmers' fields in eight locations, 2002

Line	Location [2/] 1	2	3	4	5	6	7	8
CT13941-11-M-25-5-M-M	1.07	1.08	1.17	0.96	1.20	1.52	1.21	1.36
CT13946-26-M-5-3-M-M	1.09	1.28	1.05	1.01	0.94	1.40	1.11	1.31
CT13958-13-M-17-5-M-M	1.05	1.09	1.26	0.98	1.24	1.40	1.02	1.33
CT13941-11-M-25-1-M-M	1.04	1.09	1.18	0.83	1.31	1.51	1.20	1.27
CT13956-29-M-29-2-M-M	1.08	1.34	1.01	0.93	1.04	1.27	1.14	1.02
CT13958-12-M-1-7-M-M	1.13	1.33	1.01	1.02	0.81	1.35	0.85	1.40
CT13946-26-M-5-6-M-M	1.06	1.20	1.11	0.82	1.34	1.25	0.89	1.20
Fedearroz 50	0.97	--	1.05	1.00	1.52	1.15	1.10	1.71
CT13956-29-M-14-1-M-M	1.02	1.23	1.10	0.74	1.23	1.38	1.12	1.11
CT13941-11-M-25-4-M-M	0.98	0.99	1.04	0.91	1.37	1.30	1.05	1.35
CT13958-13-M-2-3-M-M	1.06	1.13	1.16	0.82	1.19	1.02	1.18	1.04
CT13958-13-M-7-5-M-M	1.11	0.94	1.06	0.99	0.87	1.18	1.07	1.26
CT13959-3-M-10-4-M-M	1.05	1.24	0.96	0.93	0.97	1.16	0.79	1.49
CT13958-13-M-2-1-M-M	1.04	1.14	1.04	0.85	0.76	1.07	1.12	1.22
CT13956-29-M-8-3-M-M	1.02	0.94	1.08	0.94	0.94	1.24	0.80	1.40
CT13976-7-M-14-1-M-M	1.03	1.15	0.93	0.97	0.82	1.03	0.98	1.21
CT13941-27-M-19-1-M-M	0.96	1.19	0.98	0.76	1.21	1.13	1.00	1.11
CT13958-13-M-33-1-M-M	1.11	1.01	0.93	0.80	0.94	1.22	1.01	1.43
CT13941-27-M-15-3-M-M	--	1.06	--	1.04	1.53	1.16	--	1.05
CT13958-13-M-2-4-M-M	1.05	1.10	1.02	0.80	1.06	0.93	1.13	0.97
CT13958-13-M-26-5-M-M	1.07	1.00	0.94	0.87	1.09	1.01	1.11	1.30
CT13959-3-M-10-5-M-M	1.13	--	0.99	1.02	0.94	1.16	1.03	1.23
CT13956-29-M-25-7-M-M	0.99	1.06	1.10	0.76	0.77	1.27	0.94	0.88
Local checks	0.95	1.00	0.87	0.78	1.14	1.16	0.60	1.14
Bg90-2	1.00	--	1.00	1.00	1.00	1.00	1.00	1.00
CT13958-13-M-26-4-M-M	1.05	--	1.03	0.83	0.94	1.13	1.00	0.92
CT13941-27-M-5-4-M-M	--	0.94	--	0.89	1.27	1.07	--	1.33
CT13941-27-M-4-1-M-M	--	0.84	--	0.80	1.08	--	--	1.13



1/ Relative value compared to BG90-2, except for Concepción (Argentina) where values are based on El Paso 144

2/ Location 1=Aceituno 2=Concepción 3=Montería 4=Saldaña
5=Jamundí 6=Villavicencio 7=CIAT 8=Armero

Table 2. Tolerance to Several Diseases of Advanced Lines from the Cross Oryzica 3/*O. rufipogon* under Field Conditions in Saldaña, Tolima. FEDEARROZ 2002

Pedigree	**Rhizoctonia**[1]	**Sarocladium**[1]	**Helminthosporium**[1]	**Helminthosporium**[2]
CT14524-2-M-2-M	3	3	5	15
CT 14524-2-M-3-3	3	3	5	15
CT 14529-12-M-1-2	3	1-5	5	0
CT 14529-12-M-2-3	3	5	7	30
CT 14529-18-M-3-M*	3	3-5	3	0
CT 14529-18-M-4-M*	3	1-5	3	20
CT 14534-12-M-1-3	5	3	7	0
CT 14534-12-M-3-4*	3	1	1	0
CT 14534-12-M-4-1	5	3	1	0
CT 14537-8-M-4-M	3	1	1	0
CT 14537-9-M-4-1*	3	5	3	0
CT 14537-21-M-6-3	3	3	3	0
CT 14539-31-M-1-1*	3	5	3	0
CT 14539-34-M-4-M-2*	3	3	3	0
Oryzica 3 (Check)	7-9	5-7	1-3	0-20
CT 14524-3-M-2-2	7-9	7	5-7	40

[1] Scale 1-9. IRRI Standard Evaluation System
[2] % Panicle Neck Infection
***** Lines selected by FEDEARROZ

Table 3. Advanced Breeding Lines from the Cross Oryzica 3/*O. rufipogon* with Intermediate Resistance to *Rhizoctonia solani* in Greenhouse Inoculations

Line	Reaction
CT 14545-5-M-1-M-1-M	I
CT 14545-5-M-1-M-3-M	I
CT 14545-5-M-1-M-7-M	I
CT 14545-5-M-1-M-8-M	I
CT 14545-5-M-1-M-9-M	I
CT 14545-5-M-1-M-11-M	I
CT 14537-6-M-3-M-2-M	I
CT 14537-6-M-3-M-6-M	I
CT 14534-35-M-M-M-6-M	I
CT 14539-26-M-3-M-1-M	I
CT 14539-26-M-3-M-2-M	I
CT 14545-5-M-M-M-1-M	I
CT 14546-6-M-M-M-2-M	I
CT 14546-6-M-M-M-5-M	I
CT 14546-6-M-M-M-8-M	I
CT 14556-2-M-M-M-1-M	I
CT 14537-8-M-M-M-2-M	I
CT 14530-5-M-M-2-1	I
CT 14530-5-M-M-2-2	I
CT 14530-5-M-M-3-1	I
CT 14546-8-M-M-1-2	I
CT 14546-8-M-M-2-2	I
CT 14546-8-M-M-2-3	I
CT 14546-8-M-M-3-1	I
CT 14546-8-M-M-3-4	I
CT 14551-3-M-M-1-2	I
CT 14555-7-M-M-1-1	I
CT 14555-7-M-M-2-1	I
CT 14556-22-M-M-1-1	I
Oryzica 3	I
O. rufipogon	I
Fedearroz 50	S

I = Intermediate reaction
S = Susceptible reaction

Table 4. Advanced Breeding Lines from the Cross Bg90-2/*O. rufipogon* with Intermediate Reaction to *Rhizoctonia solani* in Greenhouse Inoculations

Rice Line	Reaction
CT 13941-11-M-25-4-M-M	I
CT 13941-27-M-4-1-M-M	I
CT 13941-27-M-5-4-M-M	I
CT 13941-27-M-15-3-M-M	I
CT 13946-26-M-5-6-M-M	I
CT 13956-29-M-25-7-M-M	I
CT 13959-3-M-10-5-M-M	I
Bg90-2	I
O. rufipogon	I
Fedearroz 50	S

I = Intermediate reaction

S = Susceptible reaction

Table 5. Tolerance to Rice Stripe Necrosis Virus in Doubled-Haploid Lines derived from the Cross Caiapo/*O. glaberrima* under Field Conditions. Meta, FEDEARROZ 2002

Pedigree	%Diseased Plants
1. CT16322-CA-6	2.4
2. CT16323-CA-3	5.1
3. CT16311(2)-CA-3	5.1
4. CT16318-CA-3	5.8
5. CT16308-CA-3	6.5
6. CT16322-CA-7	7.0
7. CT16313-CA-16	7.3
O. glaberrima (ACC #103544)	2.0
CG-20 (*O. glaberrima* A)	2.4
Cimarrón (Check)	40.0
O. Caribe 8 (Check)	14.0

Table 6. Tolerance to Rice Stripe Necrosis Virus in Breeding Lines derived from the Bg90-2/*O. glaberrima* Cross under Greenhouse Conditions. CIAT 2002 (data are in percentage compared to *O. glaberrima*)

Pedigree	Crinkling	Stripe/Yellowing	Stunting	Plants	
				Dead	**Healthy**
CT15150-M-21-8-2	25	40	37.5	15	39.4
CT15150-M-50-2-1	0	0	2.5	0	97.5
CT15150-M-50-2-5	0	0	0	0	100
CT15150-M-79-9-3	0	0	0	0	100
CT15150-M-79-11-2	5.3	5.3	7.8	2.8	89
CT15150-M-79-11-4	0	0	0	0	100
CT15150-M-92-3-5	22.8	35	40.3	0	59.7
CT15150-M-106-5-2	0	0	0	2.5	97.5
CT15150-M-124-1-2	2.5	2.5	2.5	0	97.5
CT15150-M-129-1-2	0	0	0	0	95
CT15150-M-129-1-3	0	2.5	5	0	95
CT15150-M-149-1-1	2.5	2.5	2.5	0	97.5
CT15150-M-181-4-1	0	0	2.5	0	97.5
CT15150-M-190-2-1	2.5	5	2.5	7.5	87.5
CT15150-M-242-3-1	0	0	0	0	100
CT16053A-6-1-1	45	55	55	10	32.5
	0	0	0	0	100
Oryza glaberrima					
Oryzica 3	55	72.5	72.5	7.5	17.5
Bg90-2	41.9	57.2	54.7	7.5	30.3
Coprosem 1	20	30	35	2.5	62.5

Table 7. Breeding Material Evaluated in Santa Rosa Experiment Station and Number of Selections made 2002

Type of Cross	No. Lines	No. Families	No. Plant Selections	Bulk Harvest
F_4 (crosses with IRRI NTP)	302	60	168	
F_3-F_4 (elite lines/wild rice lines)	405	68	180	
F_5BC_3 Bg90-2/*O rufipogon*	744	28	105	
F_4BC_3 Bg90-2/*O. rufipogon*		60	239	
F_5BC_3 Bg90-2/*O. glaberrima*	98	31	142	
F_6 (crosses with IRRI NTP)	385	129	309	
F_6 CIAT-Peru Project	1232	601	1722	246
F_7BC_3 Lemont/O. barthii	67	29	82	
F_6BC_3 Bg90-2/*O. glaberrima*	228	10	21	
F_5BC_3 Bg90-2/*O. glaberrima*	256	6	10	
F_7BC_3 Bg90-2/*O. glaberrima*	153	7	15	
F_6BC_2 Bg90-2/*O. glaberrima*	1004			694
F_7BC_2 Lemont/*O. barthii*	203	61	180	
F_6BC_2 Lemont/*O. barthii*	464	123	321	
F_8 Bg90-2/*O. rufipogon*	160			58
Recurrent Selection S_3	48	5	23	
Recurrent Selection S_4	22	19	71	
F_6 NTP (Resist. VHB)	91	19	54	
F_6 CIAT/Peru (Resist. VHB)	314	99	244	
Introduction IRRI	94	14	33*	34
No.Total Lines	**6287**	**1369**	**3883**	**1032**

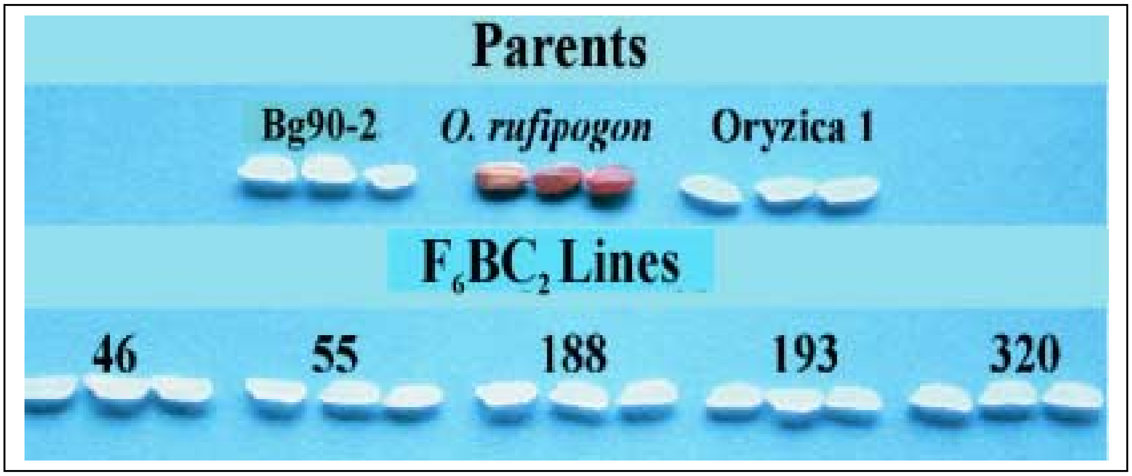


Figure 2. Advanced Lines with Excellent Grain Quality were derived from the Cross Bg90-2/*O. rufipogon*

OUTPUT 1. ENHANCING GENE POOLS

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

A. Mora and Z.Lentini

Abstract

The Anther Culture Laboratory (ACL) gives an active support to the various rice breeding efforts at CIAT by aiding the production of fixed lines through the generation of doubled haploids. This task is accomplished by the coordinated planning and evaluation between the cell tissue culture specialists and the breeders. This year report summarizes the production of doubled haploids lines and its corresponding selection for the QTL mapping of improved agronomic characteristics from inter-specific hybrids, for the development of breeding lines adapted to the irrigated tropical conditions including the introduction of the new plant type, and speeding up the release of varieties with cold tolerance of FLAR.

Introduction

Homozygous doubled haploids (DH) lines derived from spontaneous chromosome doubling of the microspore haploid genome of rice can be obtained through anther culture (AC) in less than one year, saving time in evaluation trials (DH vs. F_6) and in building up pure stocks. It is also possible to gain efficiency with DH populations when selecting for qualitative traits because of the absence of dominance, and for quantitative traits due to a greater additive variance, no intra-family segregation, and no interplant competition (Snape and Simpson, 1981; and Snape, 1989). At CIAT, AC has proved to be useful in accelerating the development of germplasm tolerant to low temperatures and having excellent grain quality, increasing the recovery of useful recombinants from wide crosses for disease and pest resistance, drought tolerance; and facilitating the production of materials suitable for molecular markers gene tagging. The CIAT rice anther culture laboratory (ACL) currently focus on developing doubled haploid lines for the various breeding efforts stationed at CIAT. In the case of CIAT, the work has been mainly directed to advanced populations adapted to the irrigated and upland savanna ecosystems, as well as backcross populations derived from crosses between cultivated rice and wild rice species. In the case of FLAR, CIAT has given a support service. The laboratory has generated lines from FLAR crosses targeting the sub-tropical and cold tolerant breeding lines pools for the Southern cone, and produced somaclone lines for Tropical Latin America. From September 2001 to September 2002, the ACL generated a total of 3,704 doubled haploids lines.

Materials and Methods

Plants were planted in the field, panicles were harvested and cold pre-treated, anthers dissected and cultured *in vitro* according to Lentini et al. (1995). Regenerated plants were delivered to the breeders to continue with the selection process under field conditions.

- ### Use of Anther Culture for QTL mapping of Inter-Specific Hybrids

Mora (IP4), G.Delgado (IP4), T. Agrono (IP4), C. Ordoñez (IP4), J. Carbalí (IP4), J. Borrero, C. Martínez (IP4, SB2), A. Almeida (SB2), C. Castaño (SB2), Z. Lentini (SB2, IP4)

Inter-specific crosses between *O. sativa* and *O. barthii*, *O. glaberrima*, or *O. rufipogon* are currently used in rice breeding at CIAT to broaden rice genetic base. These segregating lines are being evaluated for increased yield generated by transgressive segregation, and as new sources for disease resistance, and environmental adaptation. However, some of these lines are highly sterile. Anther culture is used to by-passed the sterility bottleneck and generate advanced lines for the backcross program. Doubled haploids lines derived from BCF_2 and BC_3F_1 lines from Caiapo/*O. glaberrima* crosses allowed the identification of 70 QTLs markers candidates associated with increased yield capacity of which 35 QTLs are tightly linked to the alleles derived from the wild species *O. glaberrima* (Castaño 2002).

- ### Use of Anther Culture to Advanced Breeding Populations for Tropical Irrigated Conditions

Mora (IP4), G.Delgado (IP4), T. Agrono (IP4), C. Ordoñez (IP4), J. Carbalí (IP4), J. Borrero, C. Martínez (IP4, SB2), Z. Lentini (SB2, IP4)

This year a total of 6,631 plants were generated from AC. These plants included crosses with the new plant type from IRRI, and a cross-designed to map the gene for RHBV resistance. Currently, 261 R_2 lines produced in 2000 derived from the new plant type are being evaluated for RHBV resistance, and some components of yield such as tiller capacity, days to flowering, panicle length, fertility, and grain size at CIAT experimental station, and the derived R_3 for resistance to *Pyricularia* at Santa Rosa experimental station next season. Advanced R_4 will be distributed to national programs. In 2000 it was reported a total of 318 R_2 generated from crosses with the new plant type that were selected in Palmira for plant type and yield potential. Of these, 63 R_2 lines also combined disease resistance selected at Santa Rosa Experimental Station. Three of these lines were included in the VIOAL 2001, and were evaluated in four different sites in Colombia and Argentina. In Colombian two lines were selected by the Aceituno seed company for its high yield potential, results from Argentina still had not been reported.

- **Use of Anther Culture (AC) to Accelerate the Development of Breeding Populations of FLAR**

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

In the year 2000, 21 R_3 lines generated from crosses processed through anther culture for FLAR in 1999, were selected for disease resistance at Santa Rosa and for plant type and grain quality at CIAT headquarters, and included in the VIOFLAR for the Southern Cone in 2000 (VIOFLAR 2000 included total of 175 lines, of these 12% were AC derived lines). Of the materials sent in the VIAOFLAR 2000, Uruguay selected four R_3 lines for further evaluation in 2001 for yield potential, grain quality in resistance to Pyricularia in the Station Paso de La Laguna. Results in 2002 indicated that the three lines outperformed the commercial variety El Paso 144.

Rice production in sub-tropical Latin American depends mainly on high yielding varieties such as BR-IRGA 410, BR-IRGA 417 and El Paso 144, which yield capacity derives from tropical genotypes. However, these materials are highly susceptible to low temperatures, which significantly reduce yield and their adaptation capacity. FLAR jointly with its members from the Southern Cone designed a breeding strategy to combine the high yielding capacity from tropical materials with the cold tolerance from the *japonica* rice, especially from Chilean materials. Plants from F_1's generation are first evaluated for cold tolerance under growth chamber conditions at seedling stage. Tolerant plants are then transplanted to the field, selected based on the plant type, and selected crosses are processed through anther culture. Anther culture is used here as a tool to advance the best lines, allowing to devote more effort to the most promising materials while reducing the time to develop fix lines for evaluation by the partner countries.

During the first semester of 2002 a total of 42 single crosses were processed through anther culture, generating a total of 2,594 plants from 32 crosses. Of these plants, 261 were selected and sent to IRGA, Brazil for evaluation during 2002-2003 growth seasons, while they are going to be evaluated for cold tolerance at the seedling stage at CIAT. Currently fifty-five F_1 triple crosses, which were selected for cold tolerance at the germination stage, are being processed through anther culture

- **Somaclonal Variation to Increase Genetic Variability of Advanced Breeding Lines of FLAR Member Countries**

A. Mora (IP4), G. Delgado (IP4), T. Agrono (IP4) C. Ordoñez (IP4),
E. Torres (FLAR), Z. Lentini (IP4).

In the year 2000, it was reported the generation of 3,178 somaclone lines derived from immature inflorescence using selected varieties. The goal of this activity was to induce variation for improving grain quality traits, RHBV resistance, tolerance to *Tagosodes* mechanical damage, and lodging tolerance. Last year, the S_1 seeds (first self of the original somaclone, S_0) was harvested, and S_1 plants were evaluated for grain quality and lodging tolerance, and S_2 seeds were evaluated for RHBV resistance and tolerance to *Tagosodes* mechanical damage. From these evaluations, 81 somaclones were selected (2.5%) and sent to Venezuela to be planted in the second semester of 2001. These lines were evaluated in four locations at Calabozo, Agua Blanca, Duriga and Barinas (Venezuela). Two somaclones outperformed the original varieties and were selected for increased lodging tolerance, RHBV resistance and tolerance to Tagosodes mechanical damage. These somaclones are currently being evaluated for cooking quality and yield potential.

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• Temporary Immersion System (RITA) for Anther Culture of Rice

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

Introduction

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

We have previously reported preliminary results using RITA for the induction of embryogenic callus derived rice from mature zygotic embryos (Tabares et al., CIAT SB2 Report 2000) and from anther culture (Tabares et al., CIAT SB2 Report 2001). This year we report a comparative analysis including various indica and japonica rice genotypes.

Materials and Methods

Mature zygotic embryos or anther culture of the indica rice Cica 8, Palmar, Fundarroz PN1, Cimarron, Fedearroz 2000, and CT 11275, and of the japonica breeding line CT 6241-17-1-5-1 were used. For anther culture, donor plants were grown in the field, panicles harvested, and anthers cultured according to Lentini et al., 1995. Tissues were either culture in liquid medium contained in RITA vessels or in liquid medium in baby food jars (for anthers, control) or on solid medium for zygotic embryos. Induced callus from each culture system was then transfer onto solid plant regeneration medium according to Lentini et al. 1995.

Results and Discussion

Although there was a tendency for an earlier callus induction from zygotic embryos of the indica variety Cica 8 in RITA (5 to 10 days earlier respect to solid medium), this difference was not significant (Figure 1A). However, a significant higher number of healthy calluses were induced in RITA respect to solid medium in petri dish (Figure 1B and C). A similar tendency was noted for the indica variety Palmar (Figure 2A and B). Although callus induction is enhanced in RITA, this system seems not to be appropriate for plant regeneration in rice. Green plant regeneration was highly inhibited when callus induced in RITA were also culture in liquid regeneration medium in the RITA system. In contrast, a high and similar green plant regeneration to the control was noted from callus induced in the RITA but when transferred onto solid medium in jars (Figure 3). Preliminary attempts also suggest that the induction of embryogenic callus is enhanced in RITA when a larger number of genotypes are tested, including recalcitrant indica types (Figure 4).

Future Plans

Optimize conditions for the broad application of RITA for induction of embryogenic callus in a diverse number of rice genotypes commonly used in the breeding program. Standardize conditions for an efficient use of this technique for the generation of doubled haploids from anther culture.

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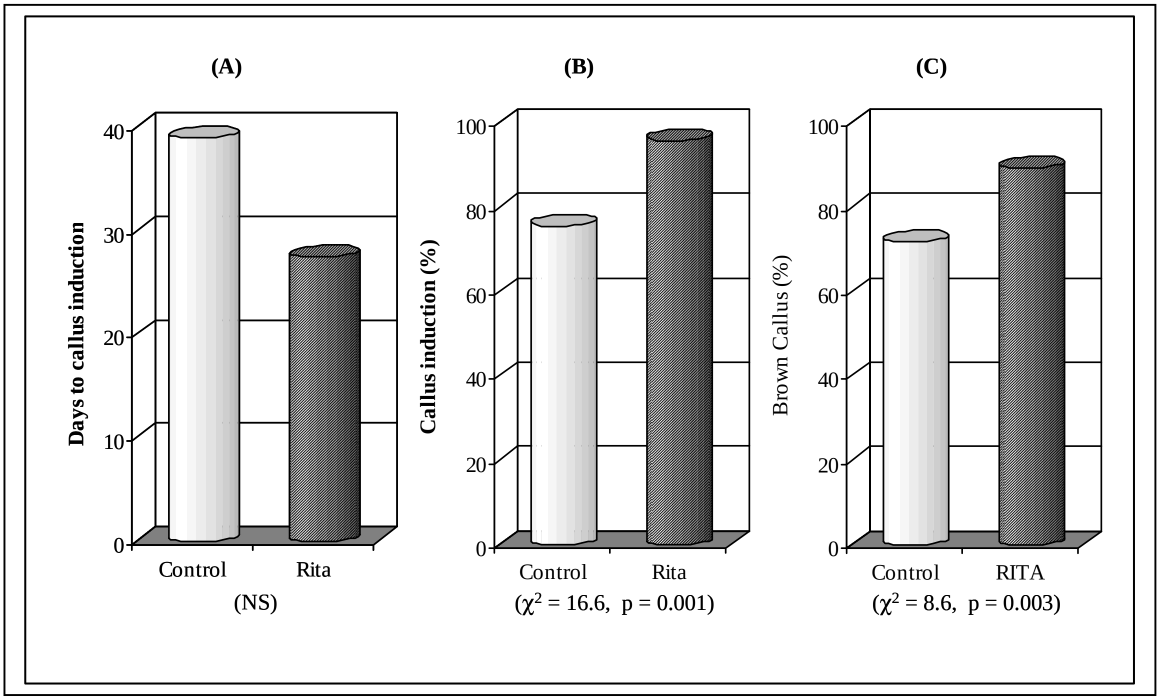
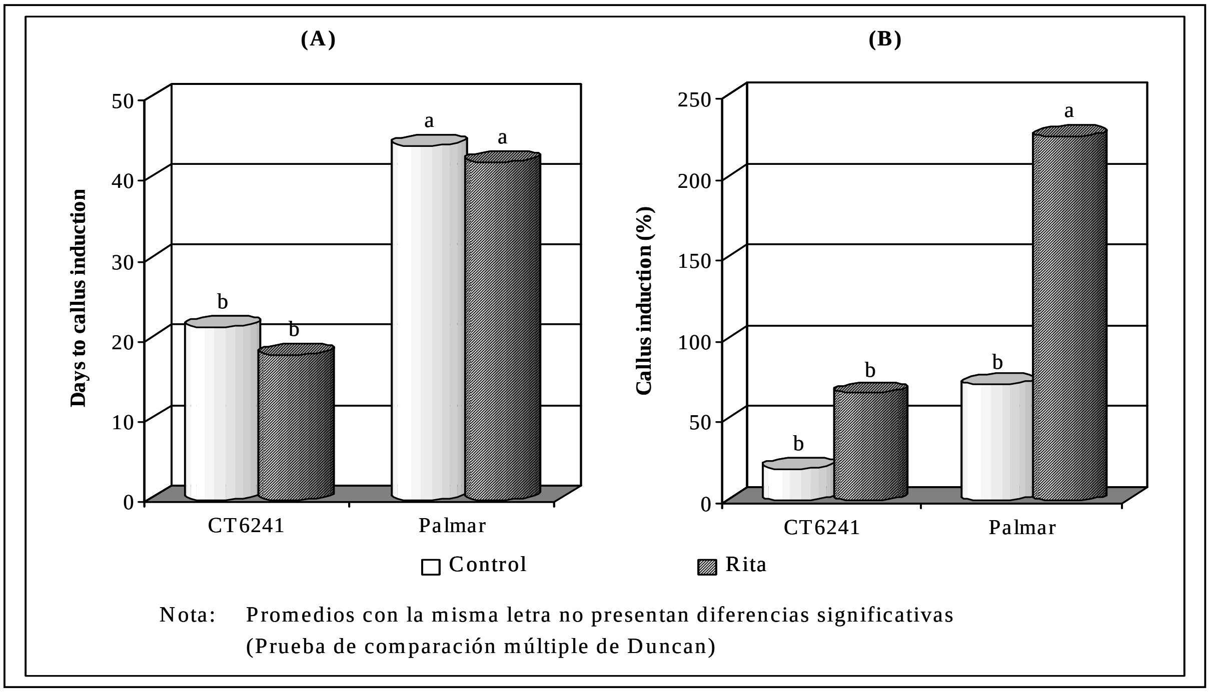


Figure 1. Callus induction in RITA System. (A) Days to callus induction. (B) Percentage of callus induced. (C) Percentage of brown (phenolized callus)



Nota: Promedios con la misma letra no presentan diferencias significativas (Prueba de comparación múltiple de Duncan)

Figure 2. Callus induction in RITA system of indica (Palmar) and japonica (CT 6241-17-1-5-1) genotypes

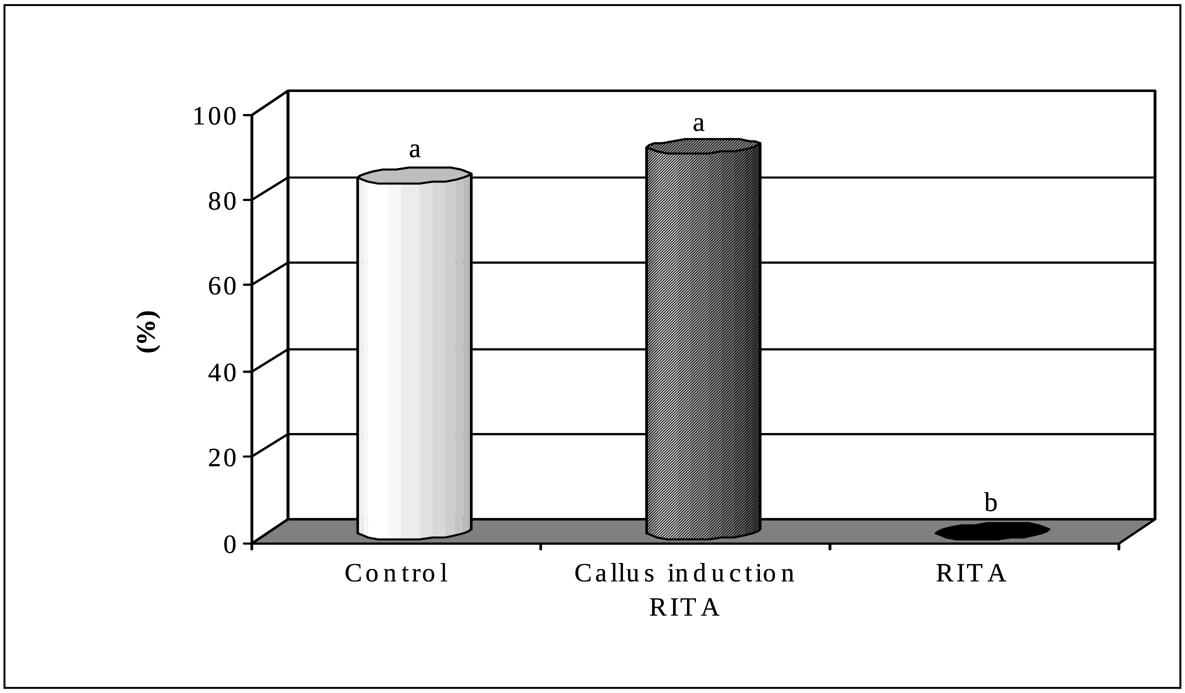


Figure 3. Green plant regeneration on solid medium in jars of callus induced in petri dish (control) or RITA, or callus induced and regenerated in RITA

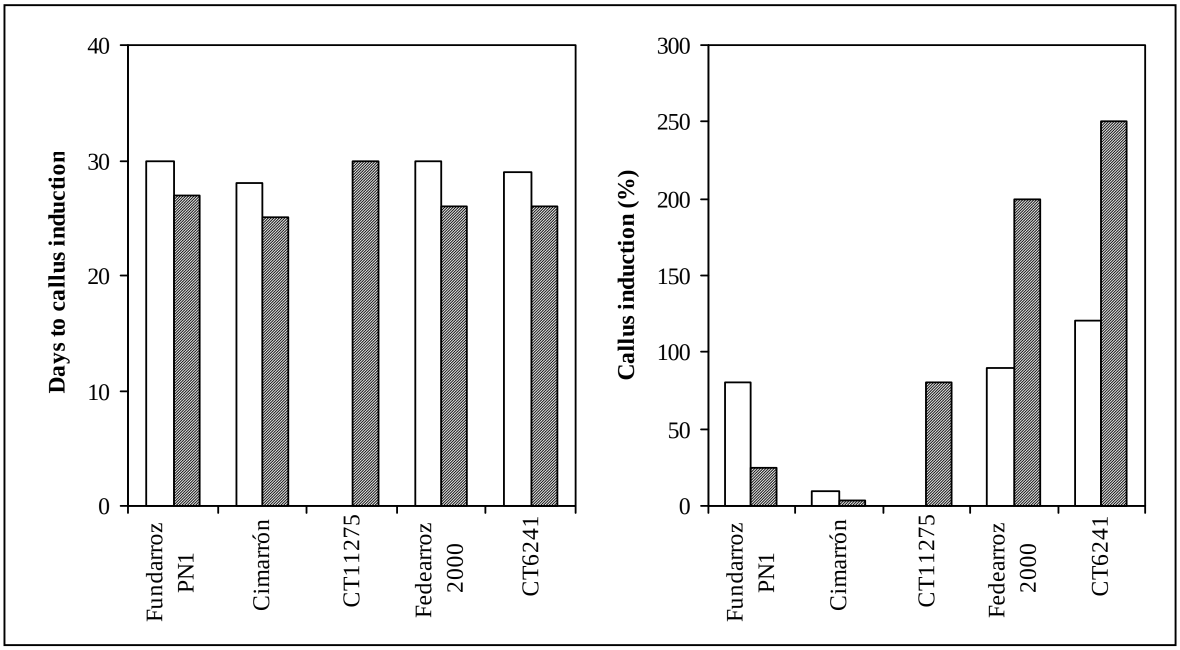


Figure 4. Callus induction in RITA of various indica and japonica genotypes

- ### Osmotic Stress as a Tool to Enhance Plant Regeneration in Rice

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[1]SB2, [2]IP4

Introduction

Between the two rice types (indica and japonica) of the most widely cultivated rice species (*Oryza sativa* L.), indica rice cultivars have proved to be less amenable to the in vitro culture. Some of the factors that affect plant regeneration frequency from rice callus are the concentrations of gelling agents, osmotic, and the specific combinations of plant growth regulators. So as to enhance green-plant regeneration, supplements such as tryptophan, proline, polyamines such as spermidine, changes in hormone composition, carbohydrate source, osmotic stress, partial desiccation and high concentration gelling agents (Khanna and Raina, 1998) have been used to improve the regenerability of rice callus culture. Partial desiccation has been reported to enhance somatic embryo differentiation and development in soybean (Hammatt and Davey, 1987), grape vines (Gray 1987), wheat (Carman 1988), spruce (Roberts et al .1991) and cassava (Mathews et al, 1993). Tsukahara and Hirosawa (1992) reported that this treatment was effective on japonica rice callus induced from cell suspension cultures. Plant regeneration frequency was considerably increased in most of the cultivars when the callus was treated with water stress, as compared with untreated controls (Lee et al., 1999). Kavi Kishor et al. (1986 and 1987) reported that the osmolarity of both growth and regeneration media was important for obtaining, retaining and reviving the high regeneration frequency of rice callus. Lai and Liu (1988) reported that the lower water content of callus cultured on a medium containing mannitol and a high concentration of agar was one of the elemental factors for efficient regeneration of rice callus. In the present study, a simple method was tested to reduce the water content of callus, thereby increasing the regeneration frequency of rice.

Materials and Methods

Three separate experiments were conducted to test different treatments to increase the plant regeneration frequency from callus culture. Experiment 1(water stress treatment): six indica rice genotypes, (Palmar, Fedearroz 50, Fedearroz 2000, Cimarrón, Cica 8, CT-11275, and Fundarroz PN1) were tested. Scutellum-derived callus of 1-2 mm diameter, were induced on the NBA medium (Li et al, 1993) medium were transferred to MS (Murashige and Skkog, 1992) semi-solid medium (0.4% agarose) or with 1% agarose (water stress), supplemented with 4mg/L Kinetin, 2mg/ l ANA, and 3 % maltose. For water stress treatments, the cultures were incubated in the dark at 27°C to dehydrate callus. After two weeks of culture, stressed callus from 1% agarose-containing medium were transferred to the corresponding 0.4% agarose solid medium for regeneration and incubated in light. The calli that were not treated with water stress were also cultured on the medium semi-solidified with 0.4% agarose Experiment two (concentration of gelling agent) after induction callus MS regeneration medium supplemented with 4mg/ l Kinetin, 2mg/l ANA, 3% sucrose, and 2g/l or 4g/l gelrite. Experiment 3 (osmotic stress), a set of callus were subcultured for 24 hr on callus induction medium +3% mannitol or 3% sorbitol, after partial desiccation treatment callus were transferred on regular plant regeneration medium. Another set of callus was transferred from callus induction medium without partial desiccation treatment to regular MS regeneration medium (control) or supplemented with either 3% mannitol or 3% sorbitol. For the three experiments a factorial experimental completely random design was used.

Results and Discussion

Plant regeneration was significantly increased in all genotypes when callus were treated with water stress for 2 weeks on medium containing 1 % agarose (Figure 5). The most significant differences were noted in Palmar, CT11275, Fedearroz 50, Cica 8, Fedearroz 2000 and Fundarroz PN1, which plant regeneration was increased from 0%-15% to 60%-100% (Figure 5). Significant differences in plant regeneration was also noted when the gelling agent (gelrite) was increased from 2 gr/l to 4 gr/l (Figure 6). Although in this case the enhancement in plant regeneration was less pronounced (Figure 6) respect to the water stress treatment (Figure 5). Pre-osmotic treatment on callus induction medium inhits plant regeneration from those callus, whereas a highly significant increased was obtained with mannitol or sorbitol was included in the plant regeneration medium (Figure 7). The best response was noted when 3% sorbitol as used to increase the osmoticum of the medium (Figure 7).

Future Plans

Comparative plant regeneration analyses of water stress treatment in callus induction and regeneration media with 1% agarose respect to increased osmoticum with 3% sorbitol in plant regeneration medium. Test best conditions with a wider range of genotypes used in rice breeding.

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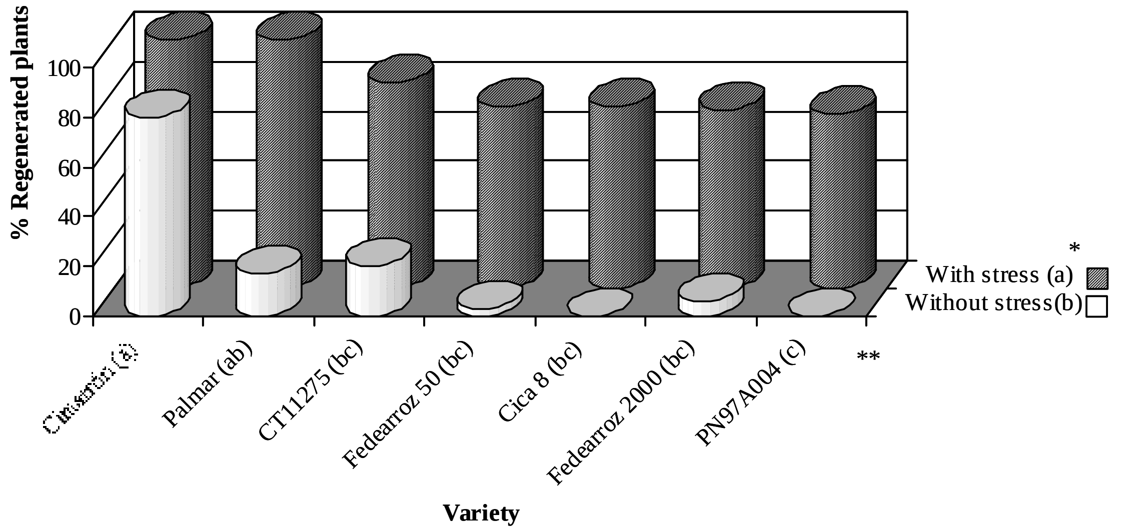

*	Comparison between treatments
**	Comparison between varieties
Note:	Mean values followed by the same letter are not significant different (Multiple Test Ryan-Einot-Gabriel-Welsch)

Figure 5. Plant regeneration of callus treated with 0.4% agarosa (without stress) por with 1% agarosa (with stress)

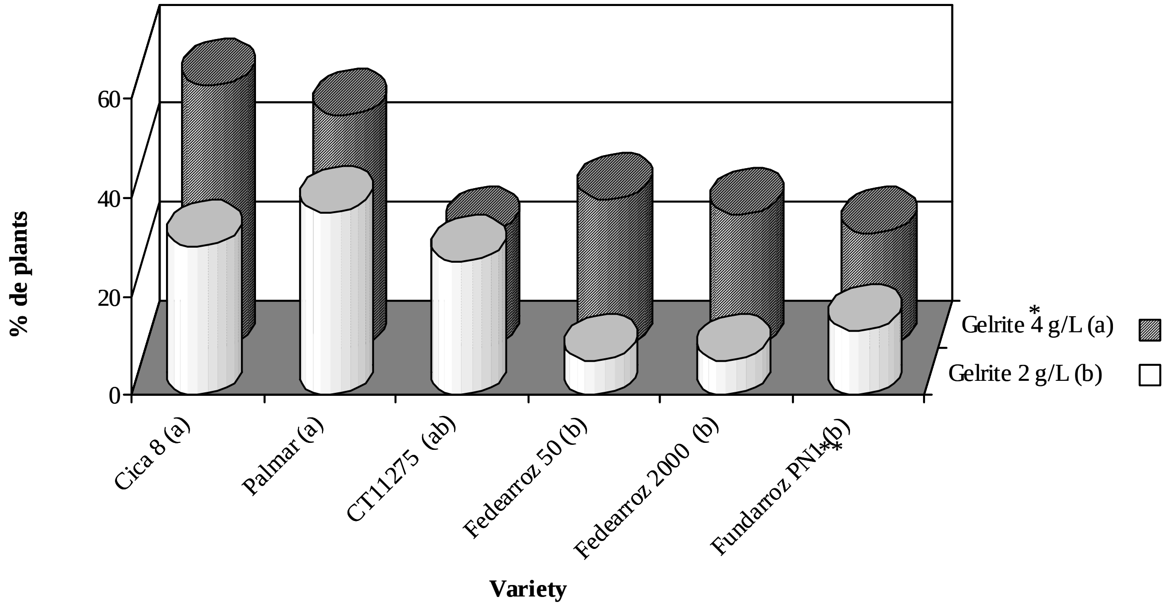

Figure 6. Plant regeneration on medium containing gelrite 2 gr/l or 4 gr/l

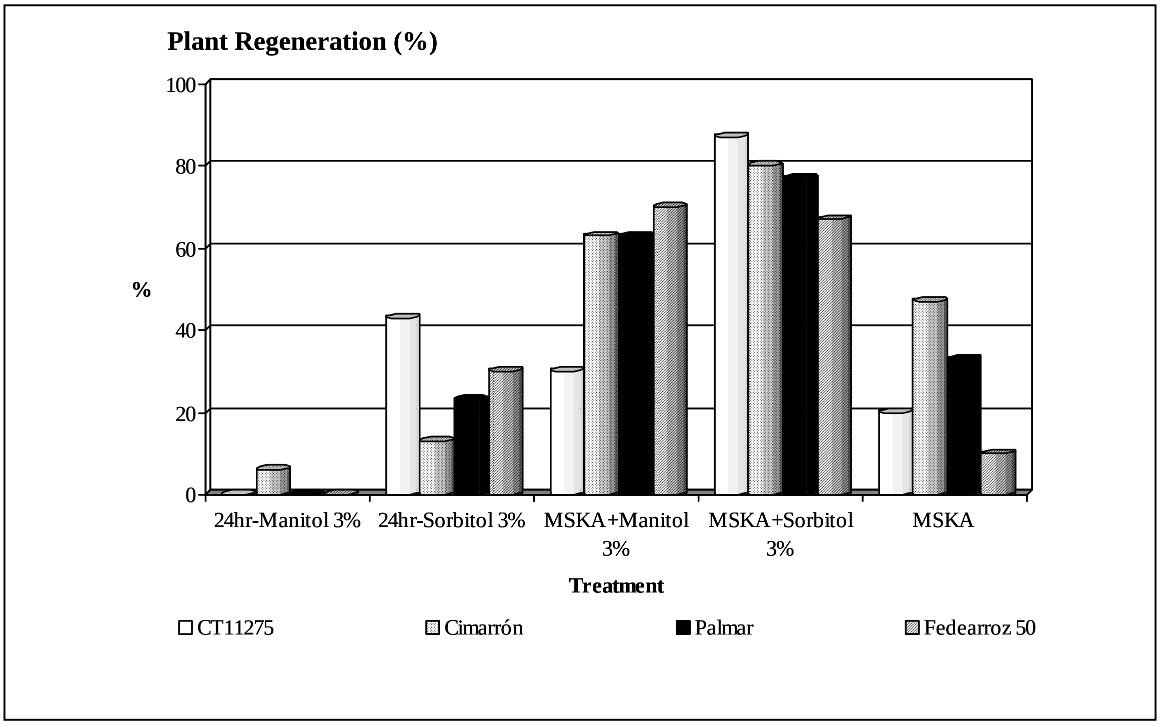

Figure 7. Plant regeneration from callus with osmoticum treatment for 24 hr or callus without treatment transferred on regeneration medium with Osmoticum

OUTPUT 1. ENHANCING GENE POOLS

1D. Gene Flow Analysis from Rice into Wild/Weedy Relatives in the Neo-Tropics

- Morphological and Phenological Characterization of Red Rice

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E. González[1], J. Silva[1], M. C. Duque[1], and Z. Lentini[1,3].
[1] SB2, [2] FEDEARROZ, [3]IP4. Funding from GTZ, Germany. Project No. 99.7860.2-001.00

Introduction

Hybridization between crops and their wild relatives sometimes brings genes into wild populations, occasionally resulting in the evolution of aggressive weeds and/ or endangerment of rare species. Transgenic crops may also result in similar outcomes. The likelihood of crop-to-wild hybridization depends on the out-cross rate, and on distance and direction between wild and crop populations. Cultivated rice, *O. sativa* L., is an autogamous plant, with a low out crossing rate of 0-1% (Roberts et al. 1961). Rice is an introduced species in the Neotropics from Africa and Asia, but with wild/weedy relatives including wild native species in Central and South America. Hybridization can be expected within the genomic group that includes *O. sativa*, viz., the AA group. The wild relatives of AA genome, which are found in Central and South America and may hybridize with the rice crop, include *O. rufipogon* and *O. glumaepatula* (Oka and Chang, 1961;Vaughan and Tomooka, 1999). Red rice (*Oryza sativa* f. *spontanea*) is weedy rice with a red pericarp and dark-colored grains. The seeds shatter readily and possess dormancy characteristics. The plants typically are tall, late maturing, and have pubescent leaves and hulls. In contrast to Asia where manual transplanting is still predominant, in tropical America direct seeding of red rice-contaminated seed source is common, making red rice the most serious weed problem. Genes from rice varieties may transfer quickly into red rice (1% to 52% hybridization rate)(Langevin et al. 1990). However, most of the hybridization rate estimates have been done under temperate conditions. This work is part of a project directed to analyze the gene flow from non-transgenic or transgenic rice into wild/weedy relatives in the Neotropics, and its effect(s) on the population genetic structure of the recipient species.

Materials and Methods

Collection of Red Rice Samples in the Field. Various commercial rice field plots were selected at Tolima, the major rice-cropping area in Colombia. Plots with known cropping history were selected. Plots had been planted with the same variety for at least the last eight growing seasons (two years), and included one of the 4 more widely grown commercial varieties: Fedearroz 50 currently grown by 80% of farmers; Oryzica 1 the previous most popular variety cultivated before Fedearroz 50 was released; and Coprosem 1 or Cimarron. A total 158 red rice plants and their corresponding seeds were harvested from each plot. Samples were grouped in populations based on their origin according to the commercial variety grown in the field. The Fedearroz 50 population is comprised by samples from different locations. Plant and seed data was collected from field collections, and a sample was stored for herbarium record. Grain colors were coded using The Royal Horticultural Society (1966): brown (code 200), greyed orange (codes 164, 166 and 173), greyed purple (codes 183 and 185), greyed yellow (code 161), and yellow white (code 158). Seeds were increased for field experiments.

Morphological and Phenological Characterization. The morphological characterization was conducted using 16 qualitative and 9 quantitative descriptors. The phenology characterization included 6 descriptors. Plant and seed morphological evaluations were conducted using the samples collected from the field (F_0 generation). The phenological characterization was performed in the greenhouse (F_1 generation) and in the field (F_2 generation) using progeny plants obtained through selfing of each F_0 plant. The corresponding rice varieties grown in the field at the moment of the field sample collection, a transgenic Cica 8 rice variety, and the wild species *O. rufipogon*, *O. glaberrima*, and *O. barthii* were used as controls.

Data Analysis. Principal component and coordinate analysis were conducted using a SAS statistical package program.

Results and Discussion

Seed parameters allowed a better characterization of the red rice populations collected. The analysis of the 1650 seeds indicated broad husk color diversity, varying from greyed yellow color (alike the commercial varieties) to brown color (alike the wild rice species). The widest diversity was noted in the populations derived from the Oryzica 1 and Fedearroz 50 plots, where about 50% of the red rice biotypes showed grains with husk color alike the corresponding commercial variety, from 2% to 15% of biotypes had husk color alike the wild rice species, and the other biotypes with husk colors corresponding to either greywed orange stripes, or brown stripes. In contrast, about 40% of the red rice biotypes derived from the Coprosem 1 and Cimarron plots showed brown grains alike the wild species, 0% and 10% of the red rice with grains alike the varieties, and only one or two of the other husk color categories. Similar patterns were noted when the awn, the apiculus, and the pericarp were characterized. Some red accessions with colored grains also showed greyed orange or greyed purple coloration in the leaves and tillers, which may facilitate the identification of hybrids since anthocyanin production in rice is encoded by dominant gene(s).

A correlation analysis between grain length and grain width also showed that the red rice populations derived from the Coprosem 1 and Cimarron field plots were the least diverse, and clearly distinct from the rice varieties. The red rice population derived from the Oryzica 1 plot was the most diverse. While some biotypes showed shorter and thicker grains alike the wild species *O. rufipogon and O. glaberrima,* others showed long and slender grains alike the varieties, and some biotypes were in between the wild species and the crop. Few of the samples from the Fedearroz 50 plot had long and slender grains. *Oryza barthii* had extra long and thick grains. Those red rice biotypes undistinguishable from the variety by their grain traits still showed seed shattering characteristic of the weedy rice.

Principal component analysis using the 9 quantitative morphological descriptors indicated that 77% of the variability could be explained by 4 main components, where grain length, width and thickness, jointly with length between nodes, and width of the flag leaf and the previous leaf were the most important. The coordinate principal component analysis using the 16 qualitative morphological parameters allowed to group the 158 red rice biotypes in three major clusters mainly characterized by the presence or absence of grain awn, and brown husk color, respectively (Figure 8). Results indicated a significant similarity between some red rice biotypes and rice varieties. Likewise a tight association was noted between various red rice samples and the wild *Oryza* species, especially with *O. rufipogon* (Figure 8). Of the phenological traits evaluated days to flowering (DTF, 50% anthesis) is the most relevant when considering likelihood of gene flow. The Ryan-Einot-Gabriel-Welsch multiple range test ($p>0.005$) discriminated three groups: early flowering (O. *glaberrima,* mean DTF 85; and *O. barthii*, mean DTF 88), intermediate flowering (all red rice accessions, rice varieties and *O. rufipogon*, mean DTF from 91 to 111), except rice varieties Cica 8 and Fedearroz 50 which were classified as late flowering according to this test (mean DTF from 112 to 116). Most red rice biotypes collected from the Fedearroz 50 plots were earlier flowering respect to this variety, but flowering of various individual biotypes overlapped with both Fedearroz 50 and Cica 8. No clear influence on the flowering pattern was noted respect to the variety previously grown in the plot (Figure 9).

Conclusions

Seed traits significantly accounted for most of the variability found in the red rice populations collected. The presence/ absence of awn and husk color grouped the biotypes in three major clusters. Seed qualitative traits jointly with the relation grain length to width easily allows to identify the red rice varietal types and those similar to wild species from the regular weedy types. Large number of red rice biotypes overlap in flowering with cultivated varieties. No clear influence in flowering pattern was noted respect to the variety previously being grown in the field. Based on this morphological and phenological characterization, some red rice biotypes had been selected to conduct gene flow analysis and identify indicators for an easy trace of gene flow in the field over subsequent generations.

Future Activities

Red rice biotypes representing the 3 major clusters with contrasting morphological characteristics easy to trace through hybridization, and overlapping in flowering respect to rice varieties were selected for evaluation to RHBV resistance.

Of these biotypes, those susceptible to the virus and easily distinguishable by red rice specific microsatellite molecular markers (described in report 1.1.12 herein) will be potential candidates for the gene flow and introgression follow up analysis using either transgenic Cica 8 resistant to RHBV or other non-transgenic variety as pollen donor.

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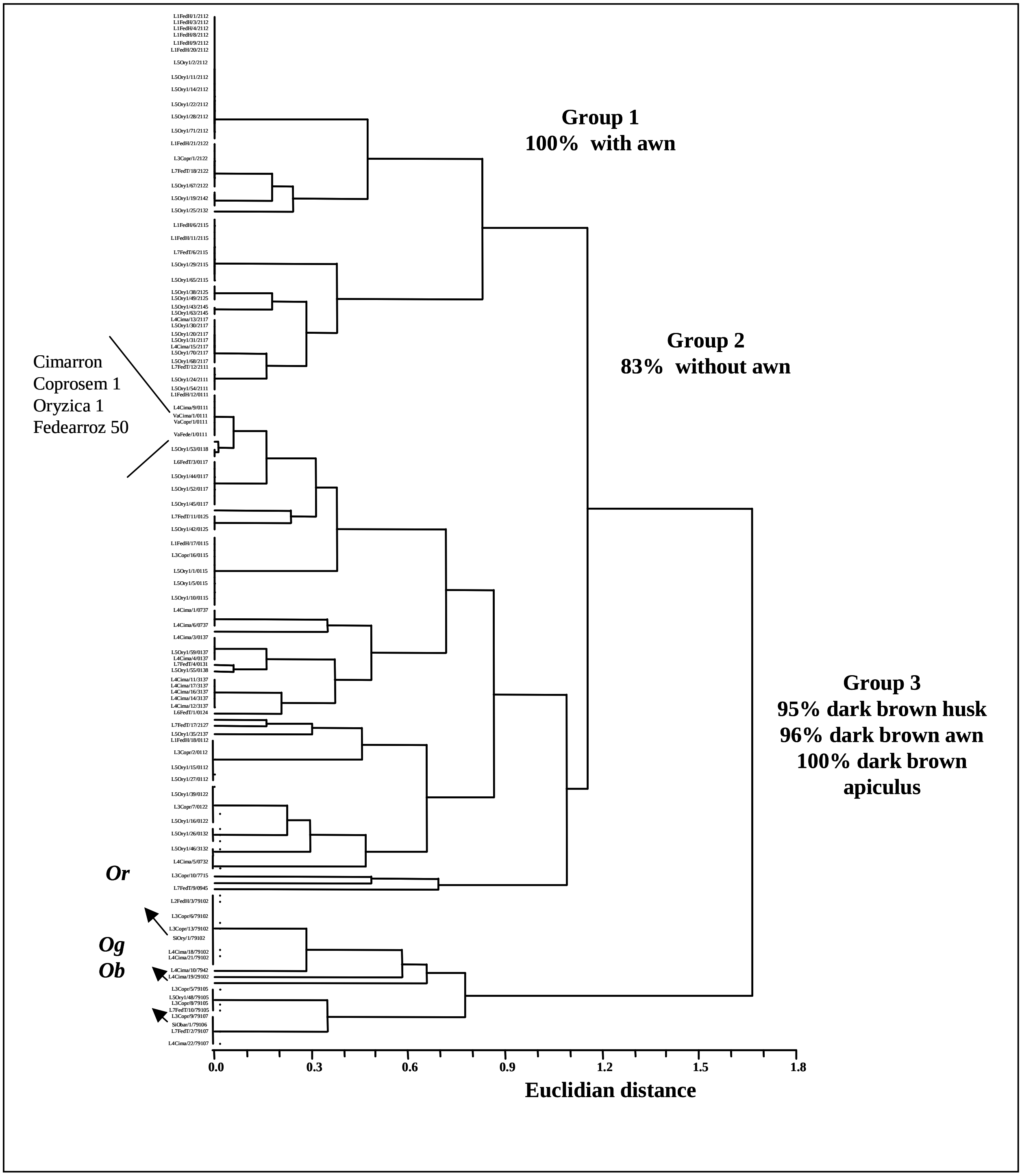

Figure 8. **Coordinate principal component analysis using 16 qualitative morphological parameters .** ***Ob* = *O. barthii;* *Or* = *O. rufipogon;* *Og* = *Oryza glaberrima***

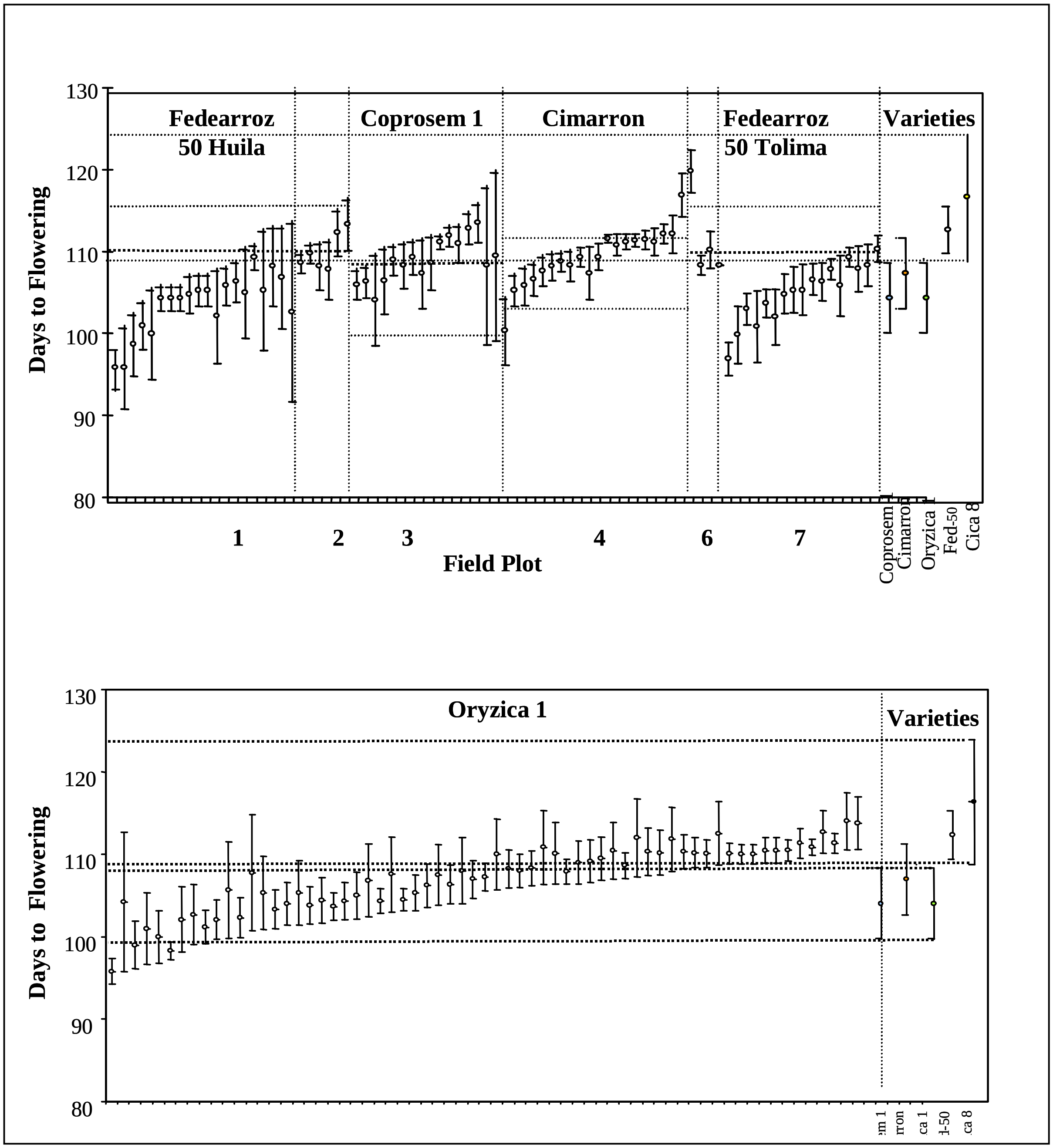

Figure 9. Days to flowering of each red rice biotype collected and corresponding variety being grown in the field at sampling time. Flowering of transgenic Cica 8 variety was included as a reference

- ## Molecular Characterization of Rice and Wild/Weedy Relatives by Microsatellites and their use to Assess Gene Flow in the Neo-Tropics

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Introduction

A careful assessment of potential impacts of gene flow from transgenic plants on population genetics of natural crop plant biodiversity is needed in other to design strategies for the safe and durable use of these crops in the Neo-tropics. This work is part of a project directed to analyze the gene flow from non-transgenic or transgenic rice into wild/weedy relatives, and its effect(s) on the population genetic structure of the recipient species. Last year we reported a preliminary screening of 50 microsatellite markers to identify those polymorphic between a set of commercial rice varieties, wild *Oryza* species, and advanced transgenic Cica 8 RHBV resistant lines and hand-made crosses with selected varieties. The current report summarizes the progress on setting up the use of microsatellites markers to assess and trace gene flow from transgenic and non-transgenic rice into wild *Oryza* species and red rice under controlled confined field plots, and under local agricultural field conditions in Colombia. A genetic diversity analysis was first conducted in order to determine the genetic structure prior gene flow, and to select the best combinations of transgenic or non-transgenic rice, and wild/weedy populations to assess the gene flow.

Materials and Methods

Plant Material. The materials included: 9 rice commercial varieties (Cica 8, Cimarron, Fedearroz 50, Fedearroz 2000, Fedearroz Victoria 1, Iniap 12, Oryzica 1, Oryzica Llanos 5, and Palmar). Sixteen homozygous transgenic-Cica 8 rice lines, resistant to RHBV virus. Four handmade crosses each between one transgenic Cica 8 line and non-transgenic Cica 8, Iniap 12, Fedearroz 50 or Oryzica 1, respectively. One hand made cross each between non-transgenic Cica 8 and Iniap 12, Fedearroz 50 or Oryzica 1, respectively (controls). One hundred and fifty eight accessions of red rice collected from various commercial rice field plots in Tolima, the major rice-cropping area of Colombia, previously characterized morphologically and phenologically. One accession each of *O. barthii, O. glaberrima, O. glumaepatula* and *O. rufipogon.* All these genotypes were included in order to select the microsatellites detecting the highest level of polymorphisms among genotypes, and the most polymorphic pairs from each class to conduct gene flow analysis.

Genetic Analysis using Microsatellite Markers. A set of 50 microsatellite markers (at least 4 per each chromosome) were screened. The microsatellite selection was based on their location in the chromosome (McCouch et al., 1997). At least two markers located distal from the centromere per each chromosome arm were chosen to increase the likelihood of finding recombination between the experimental genotypes. The markers were amplified at different annealing temperatures according to the estimated melting temperatures of the primers. The PCR products were resolved on silver-stained polyacrylamide gels and microsatellite alleles were sized by comparison to the 10 and 25 bp molecular weight standards (Promega). The genetic distance between samples was calculated using the Dice algorithm, and a dendogram was constructed using the Unweighted Pair-Group Mean Average (UPGMA). The genetic distances and dendogram were built using NTSYS-PC software version 2.02 (Rohlf, 1997).

Results and Discussion

Genetic characterization of rice varieties, red rice, and wild Oryza species. The dendrogram obtained showed thirteen groups with a similarity of 0.43. *O. glumaepatula* was the most distant group, followed by *O. glaberrima* and *O. barthii*, *O. rufipogon*, was found within the red rice group (Figure 10). For the red rice population, in some cases it was possible to associate the genetic clusters with some phenological and morphological traits. The first group was mainly composed by red rice accessions with pale yellowish husk (91%) and early to intermediate flowering (64%). The second and third groups contained accessions with grains of pale yellowish husks (95%), but with intermediate to late flowering (93%). Most of the red rice-variety biotypes and commercial varieties are in this group. The groups 8, 9,10, and 11, were mainly composed by red accessions with grain awn (69%), and intermediate to late flowering (85%). Cluster 9 grouped the wild species *O. rufipogon* with some red rice biotypes with significant similarity on morphological and phenological traits. The fourteen most polymorphic microsatellites detected a high number of alleles (106). In general the size of the alleles ranged from 108 to 252 bp. This analysis allowed the identification of 46 specific alleles for red rice; 17 specific alleles for the wild species; and only two specific alleles for the rice varieties. *O. glumaepatula* showed larger number of specific alleles (8) compared to *O. rufipogon*, which shared all their alleles with red rice. A low number of heterocigotes was found in the red rice population as a whole, with a range of 0-19 heterocygotes per microsatellite. Total genetic diversity index of Nei (0.637) was intermediate to high. In contrast, the genetic diversity index between the red rice populations collected from the different field plots was 0.55, which is considered an intermediate value. The value obtained for Gst (0.136) reflects a lower genetic diversity inter-population (collected from different field plots) than intra-population (individuals collected from the same field plot analyzed as one population).

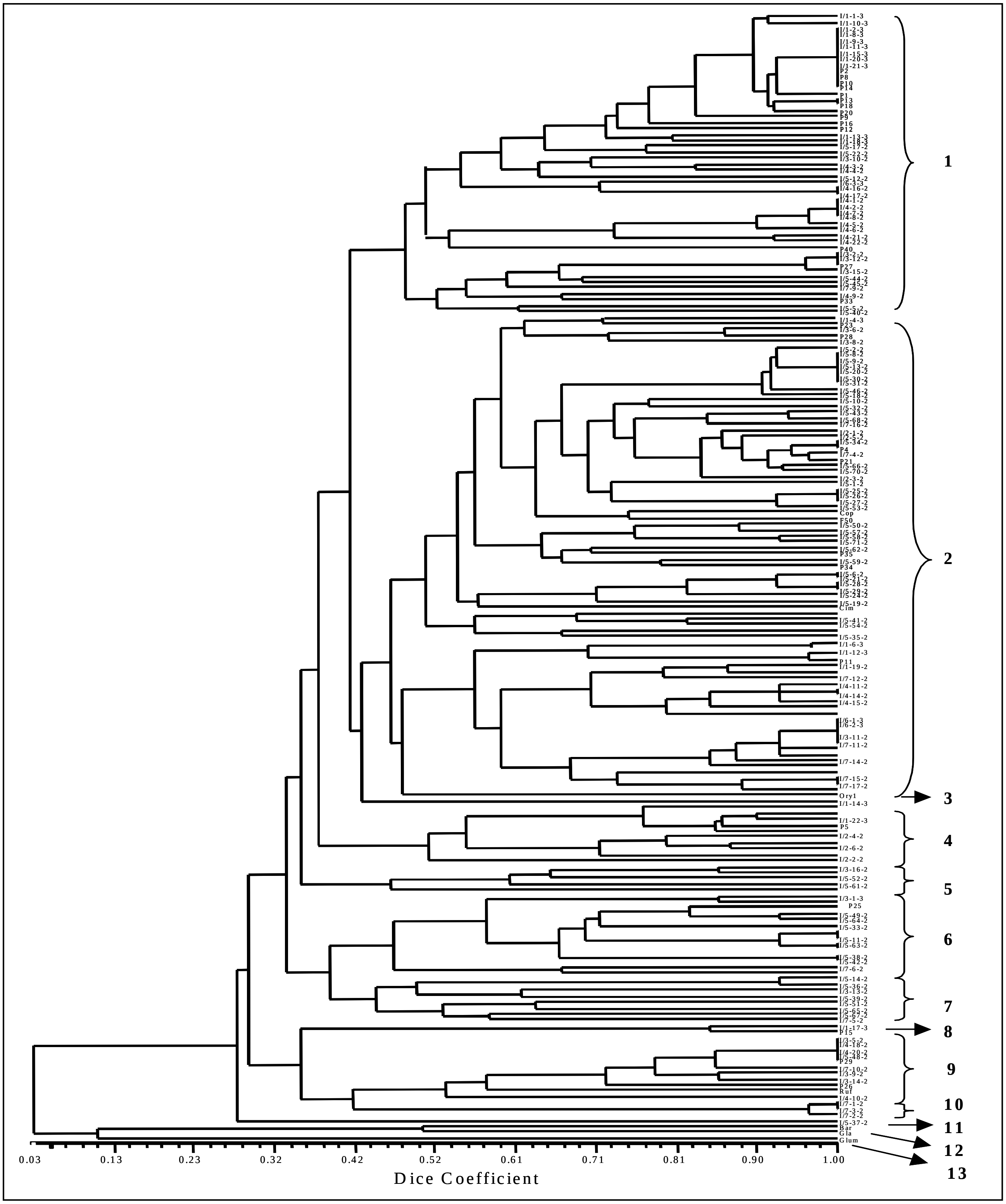

Figure 10. Genetic similarity coefficient based on microsatellite polymorphism among red rice accessions, 4 commercial varieties and d Oryza species

Tracing gene flow with microsatellite molecular markers. Clearly distinct combinations of crop, red rice, and wild species had been selected for gene flow and introgression follow up, based on the morphological, phonological, and molecular genetic characterization using microsatellite markers. Conditions had already been standardized for detection of 2% out-cross using genotype specific markers (Figure 11). Conditions are being optimized to detect 1% of out-crossing rate.

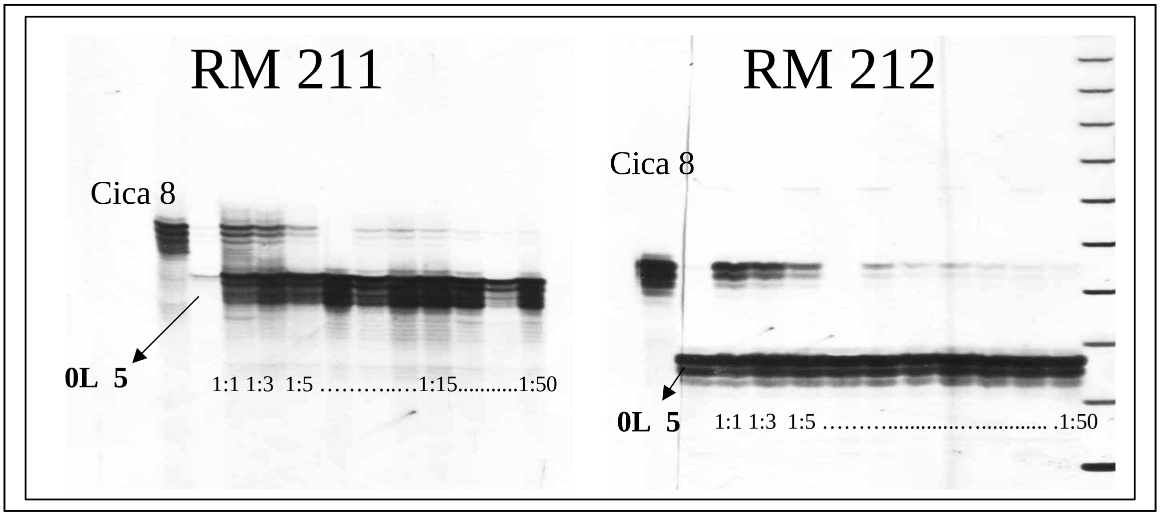

Figure 11. Microsatellite amplification of various DNA Cica 8: Oryzica Llanos 5 ratio

Conclusions

Specific microsatellite alleles were identified in different commercial varieties, red rice accessions and wild species, which can be used to trace gene flow and introgression. As expected, wild species *O. glaberrima* with *O. barthii* clustered together in a group, and in a separate cluster with *O. glumaepatula* of low similarity with the rest of the population. According to this cluster analysis, some red rice accessions showed high genetic similarity to the varieties and others to the wild species *O. rufipogon*. These genetic similarity coincide with the morphological and phenological characterization already conducted. The red rice-*O. rufipogon* biotypes will be subject of taxonomic classification to elucidate if they are introduced accessions of the wild species from Asia. The red rice-variety biotypes might be indicators of hybridization between red rice and the crop, and thus better candidates as receptors of gene flow. These materials could be ideal materials to trace transgene(s) flow. Clearly distinguishable red rice biotypes are recommended to trace gene flow from non-transgenic rice, in order to provide a broad understanding of the hybridization and introgression dynamic on this population over time.

Future Activities

Best crop/ wild/ weedy candidates to conduct gene flow and introgression analyses will be selected based on the molecular characterization and the morphological/phenological characterization presented in report 1.1.11 (herein).

Crop/wild/weedy specific microsatellites will be used to trace hybridization and population genetic through generations in the field.

The spatial distribution of alleles will be used to study local gene flow, including pollen dispersal distances.

Results will be compared with those using transgenes as a tool for tracing gene flow.

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OUTPUT 2. CHARACTERIZING RICE PESTS AND THE GENETICS OF RESISTANCE

2A. Rice Blast

- ### Characterization of Blast Pathogen Populations. Monitoring the Evolution in the Genetic and Virulence Diversity of the Blast Pathogen Over time

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Abstract

This study was initiated to understand the evolutionary response of the genetic and virulence structure of blast populations when subjected to the selection pressure of rice cultivars with resistant, partially resistant, or susceptible reaction to the blast pathogen. Breeding strategies for developing durable blast resistance will be based on the prediction of durability of blast resistance gene combinations identified on the basis of avirulence genes conserved in the different genetic groups in the blast pathogen population.

Introduction

The current experiments aim at following the changes that occur in the genetic and pathogenic (virulence and aggressiveness) structure of blast pathogen populations when subjected to selection pressure on rice cultivars with complete resistance, partial resistance, or susceptibility to the pathogen. In the field, the resistance level of the cultivars was evaluated by mean of disease ratings. Relationships between blast population changes and blast reaction of the cultivars in the field are being examined under laboratory (molecular DNA-fingerprinting) and greenhouse inoculations.

Materials and Methods

The field experiments were conducted at the Santa Rosa experiment station over a three-year period (1999-2001). Fedearroz 50 and Oryzica Llanos 5 exhibit complete blast resistance; Ceysvoni and IR 36 have partial resistance to blast; and the rice cultivars Oryzica 1 and Oryzica Caribe 8 are blast susceptible. Each variety was sown every 15 days for a total of 6 planting dates in 5x5 meter plots in each year. Evaluation of disease level in the field was done at 30, 37, 44, 51, and 58 days after sowing. For each variety and date of sowing, fifty leaves with rice blast lesions were collected 37 days after planting. Ten diseased panicles were collected for each variety and date of sowing 25 days after flowering. Genetic analysis and pathotyping was carried out for each blast sample using PCR-DNA fingerprinting and greenhouse inoculations.

Results

DNA samples of 1203 isolates of *Pyricularia grisea* collected during the three years of study were fingerprinted by using a repetitive element based polymerase chain reaction rep-PCR) with two outwardly directed primer sequences from Pot 2, an element found in approximately 100 copies in the blast fungus genome (George et al., 1998). All isolates were clustered in five genetic groups temporarily named as A, B, H, B+H, and M, and with an apparent correspondence to the previously reported SRL lineages of Colombia determined by using the MGR fingerprinting technique (Table 1). Representative isolates of each one of these genetic groups will be fingerprinted using the MGR-RFLP marker to clarify this correspondence between genetic groups. A total of 102 haplotypes were determined with group B exhibiting the largest number (43 haplotypes, B1-B43) and group M with only 1 haplotype (M1). Fifty-three percent of the isolates (641) with 22 haplotypes belonged to group A (A1-A22). Two genetic groups, A and B contained more than 80% of the collected isolates. Genetic group H had 24 haplotypes (H1-H24), and genetic group B+H 12 haplotypes ((B+H) 1-12). Genetic groups B+H and M were present in very low frequency (Table 1).

Thirteen haplotypes (974 isolates) represented 81% of the isolates analyzed (Table 2). The most common haplotype (A1) with 487 isolates represented 40.48% of the population. All other haplotypes had a frequency between 0.1 and 7.31%. The most common haplotypes in groups B (B1) and H (H10) had a frequency of only 7.31 and 7.23%, respectively. Group M had only haplotype M1 with a frequency of 2.91% and haplotypes within group B+H had frequencies of less than 1%.

Table 3 presents the number of isolates and frequency of the most predominant haplotypes per year and planting date. The number of isolates analyzed and haplotypes found per planting date was between 118 and 215, and between 31 and 61, respectively. There was no a direct relation between the number of isolates analyzed per planting date and number of haplotypes found (Table 3). For each planting date in each year haplotype A1 was the most predominant with frequencies between 34.6-53.2%. Other haplotypes that were always detected for each one of the planting dates studied were A22, A18, B1, B3, B12, and H10. Haplotypes A15 and A17 were detected in two and one planting dates, respectively. Haplotypes A1, A22, and A18 seem to group in lineage SRL-4 while haplotypes A15 and A17 within lineage SRL-5. These five haplotypes were clustered together according to the Pot-2 rep-PCR technique used in this study. Virulence groups as will be shown later exhibit different virulence patterns, suggesting the presence of two different genetic groups within the cluster A, separating haplotypes A1, A22, and A18, from A15 and A17. In general, there were no major changes in the frequency of each haplotype between the first planting and sixth planting date, with the exception of haplotypes A15 and A17, which had a major frequency in the sixth planting.

Due to the different level of resistance between the rice cultivars studied, it was not possible to obtain the same number of blast isolates for each cultivar. For some planting dates the number of isolates was low for the resistant cultivars Fedearroz 50 and Oryzica Llanos 5, as well as the partial resistant cultivar IR 36 (Tables 4, 5, 6, & 7). The number of haplotypes per number of isolates was larger for the susceptible cultivar Oryzica 1 and the partially resistant cultivars Ceysvoni and IR 36 than for the susceptible cultivar Oryzica Caribe 8 and the resistant cultivars Oryzica Llanos 5 and Fedearroz 50. The two partially resistant cultivars exhibited a larger proportion of haplotypes while the two resistant cultivars had a low proportion. For the susceptible cultivars, the number of haplotypes was cultivar dependent. There seems to be an association between the genetic group found and cultivars. For example, genetic group A was more associated with cultivars Oryzica Caribe 8 and Oryzica Llanos 5, genetic group B associated with cultivars Oryzica 1 and IR 36, and genetic group H associated with cultivar Ceysvoni (Table 4). Genetic groups A and B were recovered from the six cultivars studied. All five genetic groups were recovered from the susceptible cultivar Oryzica 1, and the resistant cultivar Oryzica Llanos 5, while only two genetic groups (A and B) were recovered from the susceptible cultivar Oryzica Caribe 8 (Table 4). The genetic group M seemed to be more associated with the resistant cultivars Oryzica Llanos 5 and Fedearroz 50 than with the susceptible or partially resistant cultivars.

The number of haplotypes per planting date per year per cultivar was in general uniform, and depended more on the number of isolates analyzed (Tables 5, 6 & 7). The number of haplotypes per number of isolates for each planting date was in general larger for the susceptible cultivar Oryzica 1 and the partially resistant cultivars Ceysvoni and IR 36. The relationship between number of haplotypes per number of isolates tended to decrease between planting dates in the same year for the susceptible cultivars, stayed similar for the partially resistant cultivars, and tended to increase for the resistant cultivars.

The frequency of genetic groups recovered from the blast susceptible cultivars Oryzica 1 and Oryzica Caribe 8 is shown in Table 8. Genetic group B was in all planting dates more associated with cultivar Oryzica 1 while genetic group A was more associated with cultivar Oryzica Caribe 8 (Table 8). Genetic group A was recovered in high frequency (30%) from cultivar Oryzica 1 on the first planting date of 1999 but was not recovered from the same cultivar on the sixth planting of the same year (Table 8). The susceptible cultivar Oryzica Caribe 8 was more specific on its susceptibility to a genetic group than the susceptible cultivar Oryzica 1 as in four planting dates 100 % of the isolates recovered belonged to group A (Table 8). The partially resistant cultivar Ceysvoni tended to exhibit more compatibility with the genetic group H (Table 9), however, in four out of six planting dates four genetic groups were recovered from this cultivar. In the first planting of year 2001, the frequency of recovery of each one of these four genetic groups (A, B, B+H, H) was equivalent, however in the sixth planting of the same year, group H predominated (Table 9). Genetic group H was predominant in four planting dates and the genetic group M was never recovered from the partially resistant cultivar Ceysvoni (Table 9). The genetic groups recovered from the other partially resistant cultivar IR 36 were predominantly B or A. In general, group B tended to predominate in the first planting of each year, however both groups tended to be in similar proportion for the sixth planting of the year. This can not be observed for the first planting of year 1999 as only 2 isolates were recovered (Table 6, Table 9). The frequency of genetic groups recovered from the blast resistant cultivars Oryzica Llanos 5 and Fedearroz 50 is shown in Table 10. Both cultivars were mainly infected by haplotypes of genetic group A in all years and planting dates, however, genetic group M also showed some specificity for these two cultivars, especially during the year 2000, and the sixth planting of cultivar Fedearroz 50 in year 2001 (Table 10). This latter group M, although was also recovered from the susceptible cultivar Oryzica 1 and the partially resistant cultivar IR 36, showed a larger specificity for the more resistant cultivars Oryzica Llanos 5 and Fedearrroz 50 (Tables 8, 9 & 10).

We are in the process of determining the avirulence/virulence gene constitution of all different 102 haplotypes found in this study to elucidate the evolutional relationship existing between the detected genetic changes and virulence spectrum of the blast pathogen. Inoculations with blast isolates representing the most common haplotypes plus (B+H)7 from in this study show compatibility with all resistance genes used, however no isolate defeated all of them (Tables 11 & 12). Haplotypes within genetic group A can be separated on the basis of their virulence spectrum in two groups as A1, A22, A18, and A15, A17 as suggested earlier based on other observations. The first subgroup has avirulence genes for the resistance genes Pi-1 and Pi-Kh, while the second group has avirulence genes for Pi-2, Pi-3, Pi-sh, and Pi-zt (Table 11). Based on the virulence spectrum observed in this study and results shown in previous annual reports of the Rice Project, we believe that these isolates represent the genetic groups SRL-4 and SRL-5, respectively. The ability to infect the resistance genes Pi-1 and Pi-33 by the haplotypes A15 and A17 suggests that these two haplotypes may belong to lineage SRL-5. Genetic group M and its only haplotype M1 had also a different virulence/avirulence spectrum corresponding more probably to the known Colombian genetic group SRL-2 (Table 11). Several resistance genes are effective against this haplotype.

Virulence spectrum of the most common haplotypes of genetic group B based on their compatibility with rice lines carrying known blast resistance genes was very similar consisting almost of one pathotype (Table 12). Avirulence genes present in this genetic group were for the resistance genes Pi-1, Pi-2, Pi-33, Pi-Kh, and Pi-Zt. Most of the haplotypes have also conserved the avirulence gene for the resistance gene Pi-ta2 (Table 12). More isolates within this group are being analyzed for determining if these avirulence genes are conserved in most isolates within the genetic group B. This genetic group, according to previous studies and comparisons is related to the previously described genetic lineage SRL-6 based on the MGR 586 RFLP markers. Haplotypes H10 and H20 within the genetic group H described in this study exhibited a different virulence spectrum to those haplotypes of genetic group B (Table 12) and tended to be more similar although no equal to the haplotypes A15 and A17 (Table 11, Table 12). This will have to be elucidated based on the molecular analysis and pathotyping of more isolates within this genetic group. Then, these haplotypes may not belong to the known Colombian lineage SRL-6 as indicated in Table 1 and may belong to the lineage SRL-5 or to a different lineage as suggested by the rep-PCR analysis using the Pot-2 primers. At least three resistance genes, Pi-2, Pi-3, and Pi-4b seem to be effective in conferring resistance to this genetic group. The virulence spectrum of the genetic group B+H represented by its haplotype 7 in Table 12 is very similar to those haplotypes of group H presented in the same Table. A more precise study is being conducted to elucidate the possible origin of this genetic group and its relation with the genetic group H and B and that of haplotypes A15 and A17. The results of this analysis will be presented in next year annual report.

Inoculation of the most common haplotypes of genetic group A on several Colombian commercial rice cultivars show similar virulence patters for haplotypes A1 and A18, but more differences between these two haplotypes and A22 compared to the inoculations on rice lines with known resistance genes (Table 11). Haplotype A22 was recovered from all six cultivars studied, however it was recovered in higher frequency from the resistant cultivars Oryzica Llanos 5 and Fedearroz 50, and specially on the sixth planting of year 2001 (data not shown). It should be however pointed out that these isolates did not infect the cultivars Oryzica Llanos 5 and Fedearroz 50 in the greenhouse inoculations (Table 11). Haplotype A1, which was recovered in high frequency from cultivars Oryzica Caribe 8, Ceysvoni, Oryzica Llanos 5, and Fedearroz 50 infected in greenhouse inoculations mainly the cultivars Oryzica Caribe 8 and Oryzica 1, but not the cultivars Ceysvoni, Oryzica Llanos 5 and Fedearroz 50 (Table 11). Haplotype A1 was recovered from cultivar Oryzica 1 in only two out of six planting dates. In fact, no isolate of any haplotype in all the five genetic groups studied infected the cultivars Oryzica Llanos 5 and Fedearroz 50 despite the fact that many isolates were recovered from the few lesions observed under field conditions (Tables 11 & 12). Inoculations of haplotypes A15 and A17 on the commercial cultivars suggest again that these isolates should belong to a different genetic group than the other A haplotypes presented in Table 13. Haplotype A15 infected the cultivars Cica 8 and Tetep, which is characteristic of the genetic lineage SRL-5 and not SRL-4. These two haplotypes presented more virulence differences when inoculated on the commercial cultivars than when inoculated on lines with known resistance genes (Table11). Both isolates however, may represent the whole virulence potential of that genetic group which seems to be different to the other A haplotypes. Genetic differences of the genetic group M are confirmed in the inoculations of haplotype M1 on the commercial rice cultivars (Table 11). This haplotype, mainly recovered from cultivar Fedearroz 50 and Oryzica Llanos 5, did not infect these two cultivars in the greenhouse inoculations. The biological significance of these observations of field infections but not observed re-infections under controlled conditions will be studied in detail with selected blast isolates from these studies and reported in the future as they might be involved in the breakdown of the resistance of these cultivars.

The genetic similarity between haplotypes within the genetic group B is supported by the inoculations of the most common haplotypes of this group on the commercial rice cultivars (Table 12). These haplotypes, mainly recovered from the susceptible cultivar Oryzica 1 infected well this cultivar in greenhouse inoculations (Table 12). On the other hand, they did not infect the cultivars Oryzica Caribe 8, Ceysvoni, Oryzica Llanos 5 and Fedearroz 50. The main characteristic of the haplotypes H10 and H20 within genetic group H, was the infection of cultivar Ceysvoni. This observation again supports the possible presence of an additional genetic lineage different to lineage SRL-6 as indicated by the use of the rep-PCR technique. The detection of this genetic group was possible due to its high compatibility with cultivar Ceysvoni in the field, compatibility that was confirmed in the greenhouse inoculations (Table 12). More isolates recovered from the cultivar Ceysvoni need to be analyzed for their pathotyping to determine if most of them infect this cultivar. Emphasis should be given to those isolates from different genetic groups recovered in the first planting of 2001 when four genetic groups were isolated from this cultivar in similar frequency (Table 9). The virulence spectrum of the haplotype (B+H) 7 is similar to those haplotypes of the genetic group H supporting the observation mentioned above that this genetic group seems to be closer to group H than to group B. This haplotype also infected the cultivar Ceysvoni (Table12).

Discussion

Despite the fact that the pathogen population studied was collected from six different rice cultivars with different levels of blast resistance (susceptible, partial resistant, and resistant), genetic group A, and particularly haplotype A1 predominated. This haplotype was recovered in high proportion from the susceptible cultivar Oryzica Caribe 8 and the resistant cultivars Oryzica Llanos 5 and Fedearroz 50. It was also significantly present on the partially resistant cultivars Ceysvoni and IR 36. A possible explanation for this interaction could be the narrow genetic base reported within the Colombian commercial rice cultivars, however, Oryzica 1, which is highly related to O. Caribe 8 and the resistant cultivars O. Llanos 5 and Fedearroz 50 showed low compatibility with this genetic group and more susceptibility to group B.

Genetic group B was also significantly important together with group A in the composition of the blast population studied. Group B exhibited however almost the double of haplotype number compared to A. The significance of this observation has to be elucidated although virulence tests realized in these studies suggest less diversity than within genetic group A. The number of haplotypes tended to be larger for the susceptible and partially resistant cultivars and lesser for the resistant. Genetic groups tended to be associated with cultivars, however this tendency was more specific for the susceptible and resistant cultivars than for the partially resistant. Although genetic group H was very specific with the partially resistant cultivar Ceysvoni, genetic group A was also recovered from this cultivar in a significant proportion. Genetic group M showed also an important association particularly with the resistant cultivars, being detected in some planting dates. In general, the partially resistant cultivar Ceysvoni showed more compatibility over the different planting dates with most of the genetic groups detected than the other cultivars, proper characteristic from this type of resistance. It should be noted however; that not all genetic groups recovered from Ceysvoni infected this cultivar in greenhouse inoculations.

Significant changes in the frequency o genetic groups recovered were observed for the partially resistant cultivars. In cultivar Ceysvoni, genetic group A was in larger frequency in the first planting than in the sixth planting. This reduction was associated with an increase in frequency of the genetic group H. In cultivar IR 36, genetic group A increased between the first and sixth plantings while frequency of genetic group B was reduced. This pattern was not observed for the other cultivars, although some genetic groups (A in Oryzica 1 and M in O. Llanos 5 and Fedearroz 50) were in some years recovered from them.

The genetic group composition of the population studied seems to represent mainly the already described genetic lineages SRL-6, SRL-5, SRL-4, and SRL-2 with the exception of genetic group H which did not fit exactly any of these lineages and could probably be another lineage or have evolved from the existing ones. Future work needs to be conducted to elucidate this hypothesis by characterizing the molecular structure of this group using different markers as well as determining the avirulence gene composition. From a breeding point of view, it is significant to note that the resistance gene combination of Pi-1, Pi-2, and

Pi-33 seems to be effective in controlling the pathogen population studied, as no isolate was compatible with these three genes. These results support our hypothesis that some avirulence genes are associated with important fitness characters of the blast pathogen and that losing them will have a high fitness penalty that could affect the viability of the fungus. Avirulence genes conserved in the pathogen, particularly in rapid evolution of this organism forced by sequential plantings of rice cultivars with different levels of resistance, could help us to predict the durability of the resistance genes.

Future Activities

Specific studies and analysis will be conducted to determine if there were changes in the avirulence/virulence gene composition of the different genetic groups and their haplotypes. It will be determined if the changes observed are associated with the selection pressure that rice cultivars included in this study with different levels of resistance/susceptibility have on the evolution of the blast population. The results of this study should help us in the development of breeding strategies for the development of durable resistance to the rice blast pathogen.

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Table 1. Genetic Group and Number of Haplotypes among 1203 Blast Isolates Collected in Evolution Studies of the Pathogen between 1999-2001

Genetic Group[1]	Number Haplotypes	Number Isolates	%
A (SRL –4/SRL-5)	22	641	53.3
B (SRL –6)	43	364	30.3
H (SRL –6)?	24	143	11.9
B + H (SRL –6)	12	20	1.6
M (SRL –2)?	1	35	2.9
Total	102	1203	100.0

[1] Genetic groups based on Pot-2 -rep PCR- DNA fingerprinting and corresponding SRL lineage

Table 2. Number of Isolates and Frequency of most Predominant Haplotypes Found in Evolution Studies of the Blast Pathogen

Genetic Group	Haplotype Identification	Number of Isolates	Frequency (%)
A	A 1	487	40.48
	A 22	55	4.60
	A 18	34	2.82
	A 15	19	1.60
	A 17	9	0.75
B	B 1	88	7.31
	B 3	71	5.90
	B 12	37	3.08
	B 34	23	1.91
	B 13	14	1.16
H	H 10	87	7.23
	H 20	15	1.25
M	M 1	35	2.91
Total	**13**	**974**	**81.00**

Table 3. Frequency of most Predominant Haplotypes per Year and Planting Date

Haplotype	Number of Isolates and Percentage											
	1999				2000				2001			
	P1[1]		P6[1]		P1		P6		P1		P6	
A 1	75	(53.2)	101	(41.7)	82	(36.3)	53	(34.6)	92	(41.4)	84	(38.4)
A 22	4	(2.8)	8	(3.3)	4	(1.8)	3	(2.0)	20	(9.0)	16	(7.3)
A 18	4	(2.8)	8	(3.3)	10	(4.4)	4	(2.6)	5	(2.3)	3	(1.4)
A 15									1	(0.5)	18	(8.2)
A 17			9	(3.7)								
B 1	15	(10.6)	28	(11.6)	15	(6.6)	14	(9.2)	11	(5.0)	5	(2.3)
	1	(0.7)	18	(7.4)	21	(9.3)	17	(11.1)	9	(4.1)	5	(2.3)
B 12	1	(0.7)	3	(1.2)	22	(9.7)	1	(0.7)	6	(2.7)	4	(1.8)
B 34			1	(0.4)	4	(1.8)	8	(5.2)	10	(4.5)		
B 13	1	(0.7)	10	(4.1)	1	(0.4)			2	(0.9)		
H 10	17	(12.1)	27	(11.2)	10	(4.4)	19	(12.4)	5	(2.3)	9	(4.1)
H 20					10	(4.4)	3	(2.0)	2	(0.9)		
M 1			2	(0.8)	17	(7.5)	8	(5.2)	1	(0.5)	7	(3.2)
Total	118	(83.6)	215	(88.7)	206	(86.6)	130	(85.0)	164	(74.1)	151	(69.0)
No. Haplotypes	32		41		48		31		61		48	

[1] P1 = First planting date; P6 = Sequential planting 75 days after first planting.

Table 4. Number of Haplotypes Recovered per Genetic Group from Blast Resistant (Oryzica Llanos 5, Fedearroz 50), Partially Resistant (Ceysvoni, IR 36) and Susceptible (Oryzica 1, Oryzica Caribe 8) Cultivars

Genetic Group	Rice Cultivars					
	Oryzica 1	O. Caribe 8	Ceysvoni	IR 36	O. Llanos 5	Fedearroz 50
A	4	12	6	6	12	2
B	29	2	9	17	2	1
H	1	0	22	3	1	0
B + H	3	0	7	0	1	0
M	1	0	0	1	1	1
Total	**38**	**14**	**44**	**27**	**17**	**4**
No. Isolates	**243**	**232**	**234**	**190**	**200**	**104**
No.haplotypes/No. Isolates	**0.16**	**0.06**	**0.19**	**0.14**	**0.09**	**0.04**

Table 5. Genetic Diversity of the Blast Pathogen Collected in Sequential Plantings of the Susceptible Cultivars Oryzica 1 and Oryzica Caribe 8

	1999		2000		2001	
Susceptible Cultivar	**P1**[1]	**P6**[1]	**P1**	**P6**	**P1**	**P6**
Oryzica 1						
No. Isolates	40	46	47	42	30	38
No. Haplotypes	13	9	13	9	17	10
No. Haplotypes/No. Isolates	0.33	0.20	0.28	0.21	0.57	0.26
Nei Index	0.79	0.75	0.78	0.77	0.85	0.84
Oryzica Caribe 8						
No. Isolates	42	48	39	36	27	40
No. Haplotypes	6	7	6	3	4	4
No. Haplotypes/No. Isolates	0.14	0.15	0.15	0.08	0.15	0.10
Nei Index	0.26	0.45	0.45	0.25	0.32	0.34

[1] P1 = First planting date; P6 = Sequential planting 75 days after first planting.

Table 6. Genetic Diversity of the Blast Pathogen Collected in Sequential Plantings of the Partial Resistant Cultivars Ceysvoni and IR36

	1999		2000		2001	
Partial Resistant Cultivar	**P1**[1]	**P6**[1]	**P1**	**P6**	**P1**	**P6**
Ceysvoni						
No. Isolates	47	48	44	29	34	32
No. Haplotypes	11	10	11	7	13	15
No. Haplotypes/No. Isolates	0.23	0.21	0.25	0.24	0.38	0.47
Nei Index	0.68	0.64	0.78	0.55	0.87	0.87
IR36						
No. Isolates	2	49	33	22	46	38
No. Haplotypes	1	7	8	6	16	9
No. Haplotypes/No. Isolates	0.50	0.14	0.24	0.27	0.35	0.24
Nei Index	0.00	0.62	0.62	0.73	.089	0.75

[1] P1 = First planting date; P6 = Sequential planting 75 days after first planting.

Table 7. Genetic Diversity of the Blast Pathogen Collected in Sequential Plantings of the Resistant Cultivars Oryzica Llanos 5 and Fedearroz 50

	1999		2000		2001	
Resistant Cultivar	**P1[1]**	**P6[1]**	**P1**	**P6**	**P1**	**P6**
Oryzica Llanos 5						
No. Isolates	10	50	40	18	48	34
No. Haplotypes	1	7	8	4	8	7
No. Haplotypes/No. Isolates	0.10	0.14	0.20	0.22	0.17	0.21
Nei Index	0.00	0.35	0.56	0.51	0.45	0.51
Fedearroz 50						
No. Isolates	0	1	23	6	37	37
No. Haplotypes		1	2	2	3	3
No. Haplotypes/No. Isolates		1.00	0.09	0.33	0.08	0.08
Nei Index		0.50	0.50	0.44	0.47	0.52

[1] P1 = First planting date; P6 = sequential planting 75 days after first planting.

Table 8. Frequency (%) of Genetic Groups Recovered from the Blast Susceptible Cultivars Oryzica 1 and O. Caribe 8 in Evolution Studies of the Blast Pathogen

	1999		2000		2001	
Genetic Group	**P1[1]**	**P6[1]**	**P1**	**P6**	**P1**	**P6**
Oryzica 1						
A	30.0	0.0	0.0	0.0	6.6	2.6
B	60.0	97.8	97.9	100.0	89.9	94.8
H	2.5	0.0	0.0	0.0	0.0	0.0
B + H	7.5	0.0	0.0	0.0	3.5	2.6
M	0.0	2.2	2.1	0.0	0.0	0.0
O. Caribe 8						
A	97.6	100.0	97.4	100.0	100.0	100.0
B	2.4	0.0	2.6	0.0	0.0	0.0
H	0.0	0.0	0.0	0.0	0.0	0.0
B + H	0.0	0.0	0.0	0.0	0.0	0.0
M	0.0	0.0	0.0	0.0	0.0	0.0

[1] P1 = First planting date; P6 = Sequential planting 75 days after first planting.

Table 9. Frequency (%) of Genetic Groups Recovered from the Blast Partially Resistant Cultivars Ceysvoni and IR 36 in Evolution Studies of the Blast Pathogen

Genetic Group	1999		2000		2001	
	P1[1]	P6[1]	P1	P6	P1	P6
Ceysvoni						
A	51.3	27.0	38.7	3.4	23.5	12.5
B	6.3	0.0	0.0	3.4	23.5	9.4
H	38.3	71.0	61.3	89.8	29.5	71.9
B + H	4.1	2.0	0.0	3.4	23.5	6.2
M	0.0	0.0	0.0	0.0	0.0	0.0
IR 36						
A	100.0	57.0	6.0	36.3	6.5	50.0
B	0.0	40.8	91.0	59.2	93.5	44.7
H	0.0	2.2	0.0	0.0	0.0	5.3
B + H	0.0	0.0	0.0	0.0	0.0	0.0
M	0.0	0.0	3.0	4.5	0.0	0.0

[1] P1 = First planting date; P6 = Sequential planting 75 days after first planting.

Table 10. Frequency (%) of Genetic Groups Recovered from the Blast Resistant Cultivars Oryzica Llanos 5 and Fedearroz 50 in Evolution Studies of the Blast Pathogen

Genetic Group	1999		2000		2001	
	P1[1]	P6[1]	P1	P6	P1	P6
Oryzica Llanos 5						
A	100.0	98.0	77.5	83.3	98.0	100.0
B	0.0	0.0	7.5	0.0	0.0	0.0
H	0.0	0.0	2.5	0.0	0.0	0.0
B + H	0.0	0.0	2.5	0.0	0.0	0.0
M	0.0	2.0	10.0	16.7	2.0	0.0
Fedearroz 50						
A	0.0	100.0	52.2	33.3	97.3	81.1
B	0.0	0.0	0.0	0.0	2.7	0.0
H	0.0	0.0	0.0	0.0	0.0	0.0
B + H	0.0	0.0	0.0	0.0	0.0	0.0
M	0.0	0.0	47.8	66.7	0.0	18.9

[1] P1 = First planting date; P6 = sequential planting 75 days after first planting

Table 11. Virulence Spectrum of Most Common Haplotypes in Genetic Groups A and M Inoculated on Rice Cultivars with Known Resistance Genes and Commercial Cultivars

Rice Line	Resistance Gene	Haplotypes					
		A1	A22	A18	A15	A17	M1
C 104 LAC	Pi-1				+++	+++	
C 101 A51	Pi-2	++		+			+++
C 101 LAC	Pi-1+Pi-33				+++	+++	
C 104 PKT	Pi-3	+++	++	+++			
C 101 PKT	Pi-4a	++	+	++	+++	++	+++
C 105 TTP4 (L23)	Pi-4b	+++	+	+++	+++		+++
F 124-1	Pi-ta	++	++	+++	+++	+++	++
F 128-1	Pi-ta^{2}		++		+++		+
F 80-1	Pi-k	++	++	+	+++	+++	
F 98-7	Pi-k^{m}	++	+++	+++	+++	+++	+++
F 129-1	Pi-k^{p}	+++	+++	+++	+++	+++	++
F 145-2	Pi-b	+	+	++	++	+++	+
Aichi Asahi	Pi-a	+++	++	+++	+++	+++	+++
K 3	Pi-k^{h}				+++	+++	
K 59	Pi-t	+	++	+++		+	++
Rico 1	Pi-k^{s}	++	+	+++	+	+	++
Nipponbare	Pi-sh	+	+	+			++
Nato	Pi-I	++	++	++	+++	+	
Ou 244	Pi-z	++	++	+++		+	
Toride 1	Pi-z^{t}	++	++	++			++
Commercial Cultivars							
Metica 1		++	++	++			+++
Oryzica 1		+	+	+			++
Oryzica 2							
Oryzica 3			+			+++	+++
Cica 4			+		+++	+++	+
Cica 6					+++	+++	+
Cica 7		++		+++			+++
Cica 8					+++		
Cica 9			++				++
IR 22			+			++	++
Tetep					+++		
Ceysvoni						+++	
O. Llanos 5							
Línea 2 (Semillano)							++
O. Llanos 4				+	+++		
O. Caribe 8		++	+	+			
O. Yacu 9		+	+				+++
SEL 320		++	+	++			++
Colombia 1		+	+	++			
Fedearroz 50							

Empty = 0 % Leaf Area Affected (LAA); + = 1-5 % LAA; ++ = 6-30 % LAA; +++ = > 30 % LAA

Table 12. Virulence Spectrum of Most Common Haplotypes in Genetic Groups B, H and B+H Inoculated on Rice Cultivars with Known Resistance Genes and Commercial Cultivars

Rice Line	Resistance Gene	Haplotype							
		B1	B3	B12	B34	B13	H10	H20	(B+H
C 104 LAC	Pi-1						+++	++	++
C 101 A51	Pi-2								
C 101 LAC	Pi-1+Pi-33						+	+	+
C 104 PKT	Pi-3								
C 101 PKT	Pi-4a	+++	+++	+++	+++	+++	++	++	+++
C 105 TTP4 (L23)	Pi-4b	+++	+++	+++	+++	+++			
F 124-1	Pi-ta	+++	+++	+++	+++	+++	+++	+++	+++
F 128-1	Pi-ta^2		+				++		
F 80-1	Pi-k	++	++	+	+	++	+++	+	++
F 98-7	Pi-k^m	+++	+++	+++	+++	+++	+++	+++	+++
F 129-1	Pi-k^p	+++	+++	+++	+++	+++	+++	+++	+++
F 145-2	Pi-b	++	++	+	++	+	+++	++	++
Aichi Asahi	Pi-a	+++	+++	+++	+++	+++	+++	+++	+++
K 3	Pi-k^h						+++		
K 59	Pi-t	+++	+++	+++	+++	++	+++	+++	
Rico 1	Pi-k^s	+++	+++	+++	+++	+++	++	++	++
Nipponbare	Pi-sh	+++	++	+++	+++	+++	++		
Nato	Pi-i	+++	+++	+++	+++	+++	+++	+++	+++
Ou 244	Pi-z	+	++	++	++	+	++		++
Toride 1	Pi-z^t						+		
Commercial Cultivars									
Metica 1		+++	+++	+++	+++	+++	+		
Oryzica 1		+++	++	+++	+++	+++			
Oryzica 2									
Oryzica 3									
Cica 4		+++	++	+++	+++	++	+++	+++	+++
Cica 6		+++	++	++	++	+++	+++		+++
Cica 7		+++	+++	++	++	+++	+		
Cica 8									
Cica 9									
IR 22		+++	+++	++	++	+++	+++	+++	+++
Tetep									
Ceysvoni							+++	+++	+++
O. Llanos 5									
Línea 2 (Semillano)									
O. Llanos 4									+++
O. Caribe 8									
O. Yacu 9									
SEL 320							+		
Colombia 1									
Fedearroz 50									

Empty = 0 % Leaf Area Affected (LAA); + = 1-5 % LAA; ++ = 6-30 % LAA; +++ = > 30 % LAA

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

F. Correa, G. Prado, G. Aricapa, M. Rubiano

Abstract

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

Introduction

The frequency of blast resistant plants observed in F_2 populations in the field is highly dependent on the blast reaction and stability of this reaction of the parents used for the development of these populations. An increase in the number of susceptible F_2 plants and F_4 lines found in the past years in different breeding materials from CIAT and FLAR at the Santa Rosa experiment station has been observed. This has been related probably to the low stability of the blast resistance of the parents used in the corresponding breeding programs. We have initiated the blast evaluation over time in the field and greenhouse of several hundred advanced as well as segregating lines exhibiting desired agronomic traits to identify potential sources of blast resistance. We are developing a nursery of potential sources of blast resistance to be used as parents, and will distribute them to partners in Latin America for testing and use in their breeding programs. We have also initiated the development of a nursery with potential donors of resistance to the brown spot, leaf scald and grain discoloration pathogens.

Results

Materials and Methods were followed according to those described in the Annual Report of the Rice Project for 2001. A total of 511 advanced rice lines from different sources (Table 13) were evaluated and selected in the Santa Rosa field experiment station in 2002. Most of the lines belong to the Germplasm Bank of FLAR from which 163 lines (71.5 %) were selected with a resistant score 0-3 to be evaluated in replicated trials in 2003. Out of 152 rice lines from three different sources (resistant lines selected in 2001, and populations 1 and 3 from recurrent selection) selected as resistant in 2001, 54.8-72.4 % (98 lines) maintained a resistant blast score 0-3 and will be evaluated again in 2003 (Table 13). New rice lines including advance lines from FEDEARROZ and some Latin American rice cultivars were evaluated and selected for their blast resistance (Table 13). In total, 301

(58.9%) lines were selected for future evaluations of the stability of their resistant blast reaction.

Table 15 shows those rice lines from different sources exhibiting complete leaf and panicle blast resistance with scores 0-2. The lines belong to different crosses from CIAT and FLAR. The Latin American rice cultivars exhibiting a high blast resistance are japonica types from Brazil (Table 15). Potential donors of resistance to leaf scald, brown spot and grain discoloration were identified with score 0-3 (Table 14). More lines will be evaluated in 2003 and the resistance of the selected lines will be corroborated under field-testing. Several of these lines are japonica types adapted to the upland acid soils with high aluminum concentration of the altillanura in Colombia. We had already reported in previous years the high resistant reaction of the japonica lines to the grain discoloration pathogens. It should also be noted the resistant reaction of the rice cultivars from Surinam Ciwini and Eloni which can be incorporated in our breeding programs as they might help to broaden the genetic diversity of our germplasm (Table 14). All these lines also exhibited a high resistant reaction to blast.

Discussion

Durability of blast resistance is in general associated with the period of time that a cultivar remains as resistant after being exposed to a targeted pathogen. Field studies conducted by CIAT at Santa Rosa demonstrated that stable blast resistance could only be identified if the lines were evaluated through the F_6-F_7 generation (Correa and Zeigler, 1995). It is possible that only after several generations of exposure that the most effective resistance genes and their combinations can be identified. These genes at the same time should correspond to those avirulence genes highly conserved in the pathogen population with lower rates of change or mutation. In order to identify resistance genes associated with durability, it is necessary to evaluate and confirm the stable resistance of the potential donors for at least seven generations. We are in the process of developing a nursery with potential donors of resistance to different pathogens. Therefore, these nurseries will be evaluated continuously for several seasons under high disease pressure in the field to assure that the resistance selected is not a escape to infection and that the lines retain their durable resistance.

Future Activities

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

References

1. Correa-Victoria, F.J., and Zeigler, R.S. 1995. Stability of complete and partial resistance in rice to *Pyricularia grisea* under rainfed upland conditions in Eastern Colombia. Phytopathology 85: 977-982.

Table 13. Selection of Rice Lines with Stable Blast Resistance for the Development of a Blast Nursery with Potential Sources of Resistance. Santa Rosa, 2002

Source	No. of Lines	Resistant		Susceptible	
		(0-3) [1]	(%)	(4-9)	(%)
Germplasm Bank of FLAR	228	163	(71.5)	68	(28.5)
Resistant Lines Selected in 2001	63	39	(61.9)	24	(38.1)
Population 1 Recurrent Selection	58	42	(72.4)	16	(27.6)
Population 3 Recurrent Selection	31	17	(54.8)	14	(45.2)
FEDEARROZ Lines	31	20	(64.5)	11	(35.5)
Latin American Rice Cultivars	86	12	(14.0)	74	(86.0)
Lines $F_6 – F_{10}$	14	8	(57.1)	6	(42.9)
Total	**511**	**301**	**(58.9)**	**210**	**(41.1)**

[1] Leaf and Panicle evaluation Scale (0-9).

- ## Table 14. Potential Donors of Resistance for Leaf Scald, Brown Spot and Grain Discoloration

Line	Leaf Scald (0-9) [1]	Brown Spot (0-9) [1]	Grain Discoloration (0-9) [1]
CT 9162-12-6-2-2-1	3	1	3
514661/Mars	3	3	1
Tres Marias	3	3	1
CT 11299-4-F_4-25P-3P	3	1	3
CIRAD 393	3	3	3
CT 8238-6-13-1P-1X	1	3	3
CT 9980-25-3-6-CA-1M	3	3	3
CT 11275-3-F_4-8P-2	3	3	3
Araguaia	3	-	1
IAC 47	3	-	1
Línea 6	3	-	1
IRAT 13	3	3	3
Ciwini	3	3	3
Eloni	3	3	3

[1] Evaluation Scale 0-9 where 0-3 = Resistant.

Table 15. Rice Lines Exhibiting Complete Leaf and Panicle Blast Resistance (Score 0-2) at Santa Rosa, 2002

Source	Line Identification
Resistant Lines Selected in 2001	CT 13503-M-3-1-M-2-1P CT 13503-M-3-1-M-2-4P CT 11891-2-2-7-M
Population 1 Recurrent Selection	CT 13449-M-8-2-M CT 13462-M-7-3-M-M CT 13465-M-6-1-M-M
Population 3 Recurrent Selection	CT 13503-M-13-1-M CT 13503-M-18-1-M
FEDEARROZ Lines	FL 00518-16P-7-3P-M
Germplasm Bank of FLAR	FL 00871-1P-5-1P-M CT 11275-3-F_4-8P-2 FL 00542-45P-8-2P-M FL 00530-7P-7-2P-M CT 11369-1-F_4-17P-4P FL 00535-21P-4-3P-M FL 00447-35P-4-2P-M FL 00459-27P-3-3P-M
Latin American Rice Cultivars	Caiapo Rio Paraguay Tres Marias
Lines F_6 – F_{10}	CT 13560-1-6-M-M-1 CT 13394-6-1-M-M-4

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

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Abstract

Farmers often choose blast susceptible varieties because they have high yields and grain quality. Since blast resistance is often broken down in a relatively short period of time, these varieties become less desirable to the farmer. Incorporation of blast resistance to these varieties would make these varieties more cost effective and ecologically sustainable. The combination of the blast resistance genes Pi-1, Pi-2, and Pi-33 confers resistance to all known blast pathogen populations of Colombia. A backcrossing program assisted by molecular markers has been initiated to introduce these resistance genes into several popular Latin American rice varieties.

Introduction

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

We have initiated a backcrossing program in order to introduce blast resistance genes into some of those susceptible rice cultivars, which still play an important role in the economy of many rice farmers and regions of Latin America. The resistance genes being incorporated into the commercial varieties are Pi-1, Pi-2, and Pi-33 as they confer resistance to all the pathogen population in Colombia and probably the Latin America region based on their reaction to other blast populations of the region. It should be clarified here that the resistance gene Pi-33 corresponds to the resistance gene named as Pi-11 in previous reports of these studies, and the change has been made according to the present classification of the blast resistance genes. The BC_1F_1 breeding populations between several Latin American rice varieties and four rice lines as sources of the three resistance genes have been developed for the identification of heterozygous lines carrying the three resistance genes. Materials and Methods were described in the 2001 Annual Report of the Rice Project.

Results

Near isogenic lines carrying any of the three resistance genes, combination of any two genes, and combination of the three resistance genes Pi-1, Pi-2, and Pi-33 were developed based on controlled inoculations in the greenhouse and evaluated under field conditions in

2002 in our Santa Rosa experiment station. Four lines carrying the three resistance genes were included in the field evaluations. The only isogenic lines exhibiting a leaf and panicle blast resistance were the four lines carrying the three resistance genes Pi-1, Pi-2, and Pi-33 (Table 16, Figure 1). Most of the lines carrying one or two genes died before reaching panicle development. Isogenic lines carrying only one of the genes were severely defeated by the pathogen. The combination of the resistance genes Pi-1 + Pi-33 exhibited also a highly susceptible blast reaction. Some isogenic lines carrying the resistance gene Pi-2 in combination with Pi-1 or Pi-33 exhibited some level of tolerance to blast. The isogenic line carrying the resistance genes Pi-1 + Pi-2 was highly tolerant and more work will be carried out to determine if the level of resistance observed is due only to the presence of these two genes, or if any other gene including Pi-33 could be present in the line.

The four isogenic lines carrying the three resistance genes Pi-1, Pi-2, and Pi-33 with a high leaf and panicle blast resistance (Table 16) were used for the development of the BC1F1 populations of fourteen Latin American rice varieties following the procedure described in Figure 2. Molecular markers (microsatellites) associated with the blast resistance genes were identified (Figure 2) for the identification and selection of resistant plants carrying the three resistance genes for the production of the BC_2F_1 populations. Greenhouse inoculations with appropriate blast isolates will be made for corroborating the presence of the three resistance genes. The number of backcrosses will depend on the recovery of the desired agronomic traits of the recurrent rice varieties.

Discussion

The combination of the three resistance genes Pi-1, Pi-2, and Pi-33 in a single near isogenic line exhibited complete leaf and neck blast resistance when exposed under high blast pressure in a blast pathogen population with pathotypes compatible with the combination of any two of these three resistance genes. It seems then that the blast pathogen is able to lose any of the three avirulence genes or any combination of two avirulence genes, but not the three of them in a single isolate. Preliminary observations under controlled inoculations in the greenhouse did not indicate a direct association between the lost of an avirulence gene in the pathogen with fitness parameters easily measured in these studies such as sporulation, leasion size, or leaf area affected using near isogenic lines carrying none of the three resistance genes or combinations of two genes. It is possible that losing avirulence genes for one of these three resistance genes might affect other fitness parameters such as competitiveness among pathotypes with different avirulence gene composition, and therefore this parameter needs to be measure under natural conditions of infection by collecting blast samples from different near isogenic lines carrying different combinations of the resistance genes.

Future Activities

Evaluation and selection of the first BC_1F_1 population of several backcrosses between Latin American rice varieties and near isogenic lines carrying the resistance genes Pi-1, Pi-2, and Pi-33 for the selection of rice lines with the three genes for the production of the BC_2F_1 populations. Molecular markers as well as inoculations with appropriate blast isolates will be used for the identification of the rice lines carrying the three resistance genes. Field studies will be carried out to determine the possible association of the lost of an avirulence gene with parameters of pathogenic fitness such as pathogen competitiveness. These activities will be carried out by collecting blast isolates from near isogenic lines with different combinations of the three blast resistance genes and comparing the frequencies of the different avirulence gene combinations present in the pathogen population with the expected frequencies based on the resistance genes of each line.

Table 16. Leaf and Neck Blast Reaction of Rice Lines with different Combinations of the Resistance Genes Pi-1, Pi-2 and Pi-33

Rice Line	Resistance Gene	Leaf Blast BL4[1]	Leaf Blast BL5[1]	Neck Blast Incidence (%)
CT 13432-95	None	9.	9	Dead
CT 13432-110	None	9	9	Dead
CT 13432-68	1	9	9	Dead
CT 13432-68	1	9	9	Dead
CT 13432-54	2	9	9	Dead
CT 13432-219	2	8	6	35
CT 13432-6	33	9	9	Dead
CT 13432-33	33	9	9	Dead
CT 13432-230	1 + 2	9	9	Dead
CT 13432-66	1 + 2	4	3	0
CT 13432-234	1 + 2	6	4	10
CT 13432-26	1 + 33	9	9	Dead
CT 13432-62	1 + 33	9	9	Dead
CT 13432-7	2 + 33	8	6	10
CT 13432-96	2 + 33	7	4	0
CT 13432-193	2 + 33	8	5	15
CT 13432-200	2 + 33	7	5	0
CT 13432-34	1 + 2 + 33	3	2	0
CT 13432-107	1 + 2 + 33	2	1	0
CT 13432-189	1 + 2 + 33	2	1	0
Ct 13432-246	1 + 2 + 33	2	1	0

[1] Fourth and Fifth Evaluations of Leaf Blast.

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

F. Correa and M. Rubiano

Abstract

Blast resistance is commonly lost in breeding lines after the F_4 or more generations. The first selections for blast resistance are made by breeders in early generations (F_2), however, it is not known the association of stability of resistance of the selected plants and the original blast reaction of an F_2 population. A long-term study was initiated in 2000 to associate the stability of blast resistance in advanced generations and the reaction of F_2 populations. We expect to generate information for selecting resistant plants in early generations that would potentially have more chances of leading to the identification of rice lines with stable blast resistance.

Introduction

It is commonly observed that blast resistance is lost in breeding lines selected for resistance after four or more generations. Stability of blast resistance under severe blast pressure is the result of the action of many resistance genes. Many strategies and breeding methodologies for the selection of rice lines with stable resistance have been tried. Normally the first selections for blast resistance are made in the F_2 generation, but the stability of resistance selected at this early stage is not known. We have initiated a long-term study to determine the association of stable blast resistance and the original blast reactions observed in the F_2 population. This is being done using advanced lines developed in a breeding program, where parents and crosses are selected on the basis of their reaction to blast lineages and/or field reaction. Studies of the stability of the blast resistant reaction will continue with the advanced generations in replicated trials until the number of lines losing the resistance reaches a plateau. Materials and methods have been described in the Annual Report for 2001 in the Rice Project.

Results

A total of 292 F_2 blast resistant plants from 87 different crosses and 147 families were selected in year 2001 (Table 17). The resistant plants were selected from crosses where the predominant F_2 family field reaction was susceptible, segregating (equal amount of F_2 susceptible/resistant plants), or resistant F_2 plants (Table 17). Evaluation of the F_3 lines derived from the F_2 resistant plants selected in 2001 yielded more resistant lines (35.5%) when selections were made from the F_2 populations that were rated as resistant to blast compared to those coming from segregating or susceptible. There were apparently no differences between these two latter groups, which yielded 6.7 and 8.7 % of resistant F_3 lines (Table 17). The segregating F_2 family yielded more lines with an intermediate blast reaction than the susceptible F_2 families. Resistant F_4 plants within each one of these three groups of lines were made in 2002 and the lines will be evaluated in replicated trials in

2003. These results are in accordance to those reported in last year report for the selections made in 2000.

A total on 1017 F_4 lines derived from F_2 resistant plants selected in 2000 were evaluated in 2002 in replicated trials (Table 18). The largest proportion of resistant lines (50.5%) originated from those lines coming from F_2 populations where the number of resistant plants predominated. However, this proportion was highly similar to the proportion of resistant lines coming from segregating F_2 populations (48.2%, Table 18). It is important to point out that a significant number of resistant lines (30.1%) were coming from susceptible F_2 families, however, the stability of this resistance should be evaluated in the future and compared with that coming from the other two groups. The number of resistant lines derived from crosses where the three parents had a resistant blast reaction tended to increase in the F_4 generation (35.4%) compared to the F_3 generation (23%, Table 18). Few plants within each one of the 1017 lines were harvested and will be planted as F_5 lines in 2003 together with the F_4 lines coming from the trials started in 2001. Evaluation of the stability of the resistance of these advanced lines will continue on a yearly basis in replicated trials.

Discussion

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

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

Future Activities

Analysis of the blast reaction of the parents involved in all crosses that gave origin to the rice lines of this study will be carried out and correlated to the blast reaction and stability of the resistance of the lines being selected. This study is in progress and will continue for several years for determining their stability.

Table 17. Long Term Study on the Stability of Blast Resistance based on F_2 Resistant Plants Selected from different Rice Populations in 2001. Santa Rosa, Colombia

				Evaluation of F_3 Lines in 2002					
	Crosses	Families	Resistant	Resistant		Intermediate		Susceptible	
Population	(No.)	(No.)	F_2 Plants[1]	No.	(%)	No.	(%)	No.	(%)
F_2 Family Field Reaction									
Susceptible	42	50	105	7	(6.7)	37	(35.2)	61	(58.1)
Segregating	45	49	92	8	(8.7)	48	(52.2)	36	(39.1)
Resistant	36	48	95	34	(35.8)	48	(50.5)	13	(13.7)
Total	87	147	292						

[1] Selected in 2001

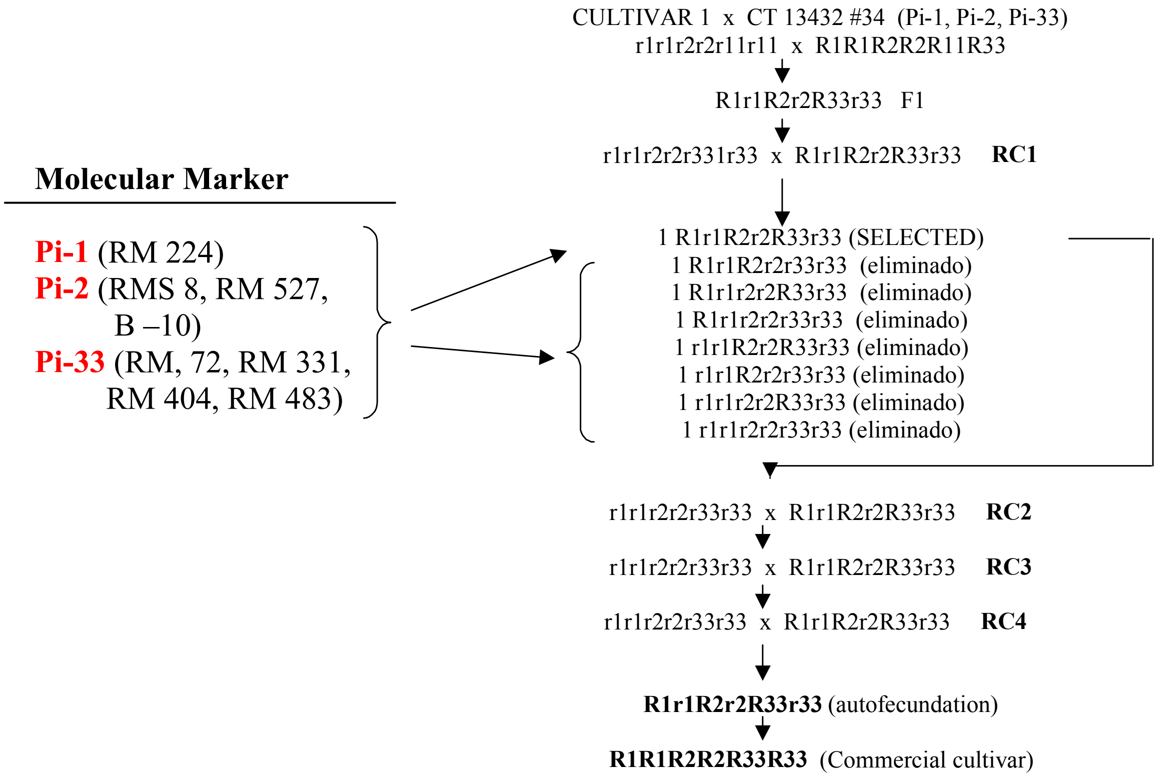
Table 18. Long Term Study on the Stability of Blast Resistance Based on F_2 Resistant Plants Selected from different Rice Populations in 2000. Santa Rosa, Colombia

Population Cross/Family	Crosses (No.)	Families (No.)	Resistant F_2 Plants* (No.)	Evaluation of F_3 Lines in 2001						Resistant F_3 Lines** (No.)	Evaluation of F_4 Lines in 2002			
				Resistant		Segregating		Susceptible			Resistant		Susceptible	
				No.	(%)	No.	(%)	No.	(%)		No.	(%)	No.	(%)
Progenitors Selected														
R/R//R	13	27	53	12	(23)	34	(64)	7	(13)	164	58	(35.4)	106	(64.6)
S/S//S	8	15	28	1	(4)	1	(4)	26	(92)	92	2	(2.2)	90	(97.8)
F_2 Family Field Reaction in 2000														
Susceptible	27	50	89	32	(36)	37	(42)	20	(22)	289	87	(30.1)	202	(69.9)
Segregating	27	47	79	45	(57)	27	(34)	7	(9)	276	133	(48.2)	143	(51.2)
Resistant	18	30	60	46	(77)	12	(20)	2	(3)	196	99	(50.5)	97	(49.5)
Total	**69**	**169**	**309**							**1017**				

* Selected in 2000
** Selected in 2001

Figure 1. Complete Blast Resistance of near Isogenic Line CT13432-34 Conferred by the Combination of Resistance Genes Pi-1+Pi-2+Pi-33

Figure 2. Backcross procedure for the Introgression of the Resistance Genes Pi-1, Pi-2, Pi-33



OUTPUT 2. CHARACTERIZING RICE PESTS AND THE GENETICS OF RESISTANCE

2B. Characterizing and Using Resistance for the Control of Rice Blast Disease

- **Recurrent Selection to Improve Partial and Complete Resistance to Rice (*Oryza sativa*) Blast (*Magnaporthe grisea*) Disease and Other Agronomic Traits in the Population PCT-6 of *indica* Lowland Rice**

M. Valès, J.P. Dossmann, F. Rodríguez, V.H. Lozano, J. García, M.C. Duque, J. Silva

Abstract

A scheme of recurrent selection is being tested to enhance populations for complete and partial resistance to rice blast disease, pest tolerance, and other agronomic traits. A second selection cycle of recurrence in *O. sativa* sub-sp *indica* lowland rice population PCT-6 was made. The second selection for partial resistance to rice blast disease, pest tolerance, and other agronomic traits allowed an evaluation of genetic progress obtained through the first complete cycle of this new recurrent selection scheme. For this report the analysis in focused on the gain of production and on the progress for blast resistance. With 19 kg of seeds / ha the production of the better lines progress from 6.750 to 7.750 t/ha. All the productive lines are resistant to blast. Nevertheless the progress for blast resistance is not clear. It is may be due to the difficulty for analyzing the data cause by the interaction between the strains and the checks for partial resistance. Other explanation: the strain used in 2002 is more aggressive than the strain employed in 1998.

Introduction

Despite many selection efforts for durable rice blast resistance, most commercial varieties become susceptible in 1 to 3 years after introduction. We are attempting associate blast resistance genes for complete and for partial resistance in enhanced populations. Recurrent selection is an appropriate method to enhance polygenic characters in breeding populations. For this reason this method is proposed for the accumulation of resistance genes to rice blast disease. To facilitate the genetic recombination a recessive gene of male sterility is used (Singh and Ikehashi, 1991).

The scheme of recurrent selection (Vales, Rice Project Annual Report 1998) included the selection of complete resistance, the selection for the partial resistance as well as important agronomic characters. The method involves genetic recombination that maintains the population's variability and maintains a high frequency of the male sterile gene in order to allow further cycles of recurrent selection (Figure 1).

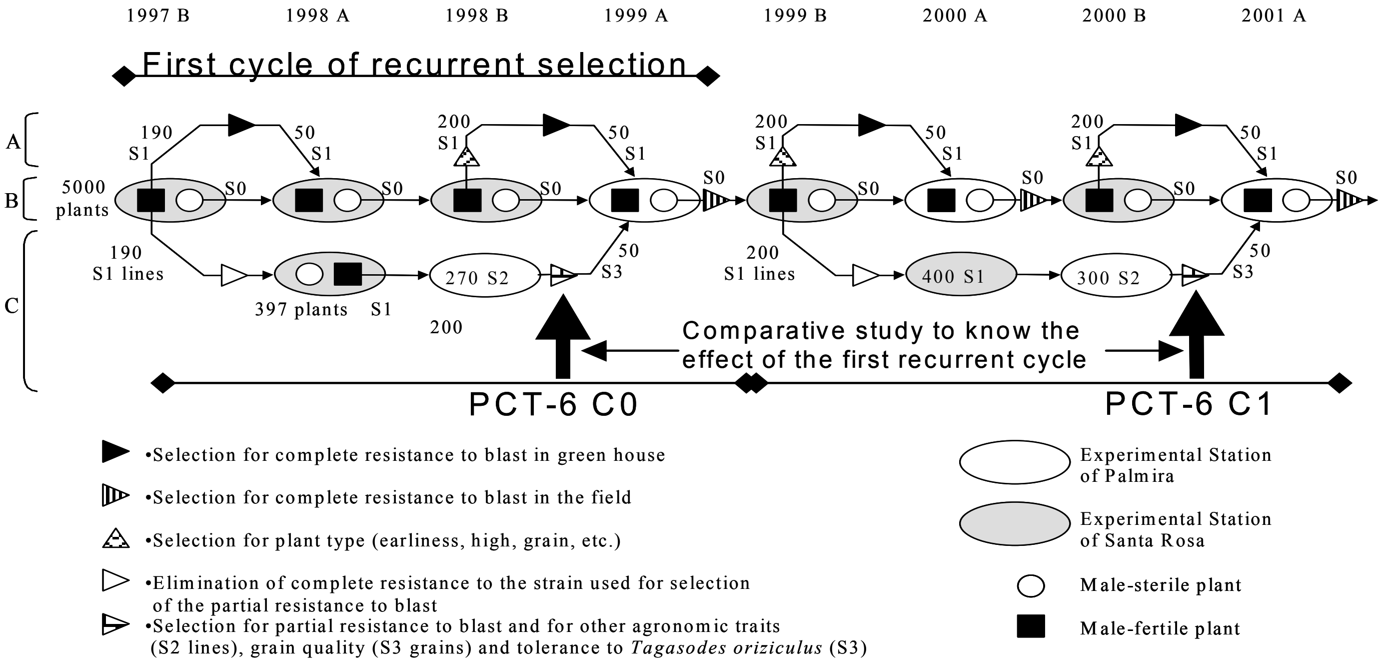


Figure 1. Recurrent Selection in the PCT-6 Population for Partial and Complete Resistance to Blast and for other Agronomics Traits

Materials and Methods

The population PCT-6 of *O. sativa* sub-sp *indica* upland rice went through two cycles of recurrent selection for rice hoja blanca virus (RHBV). Additional cycles of recurrent selection were then done following the scheme shown in Figure 2 (Vales, Rice Project Annual Report 2001). The origin of the material used in the trials is described in the follow paragraphs.

The recurrent selection scheme used to develop the population PCT-6: (A as first semester, B as second semester).

In 1997B and 1998B, there were two cycles of selection in S_1 lines under greenhouse conditions. The genetic progress of complete resistance to rice blast due to the first selection cycle was considerable, with an important increase in the percentage of S_1 lines resistant to strains of the different rice blast lineages. During 1999, a cycle of selection for rice blast in field conditions using the S_1 plus S_3 population.

In 1998A, a cycle of recombination in S_1 plus S_0 was made, after the selection in greenhouse for the complete resistance in S_1.

In 1998B, selection of S_2 lines for the partial resistance to rice blast disease, yield, precocity, plant type, and resistance to *Diatraea saccharalis* was done in field experiments. The S_3 grain was tested for quality and the S_3 plants were evaluated for tolerance to *Tagosodes orizicolus* (Valès, Rice Project Annual Report, 1998).

In 1999A, a cycle of recombination in S_1 plus S_3 and the incorporation of S_0 was made after the selection in greenhouse for the complete resistance in S_1 and selection for partial resistance in the field in S_2 and other agronomic traits in S_2 and S_3.

In 1999B, the S_0 obtained with the recombination was planted. A selection was made for high inheritance characters using 5000 plants in half-S_1B lines. The seeds from 300 selected male-fertile plants were harvested and became the S_1 lines.

In 2000B, seeds of S_2 lines, without complete resistance to specific strains, were obtained. This material was used in the selection for the partial resistance to rice blast disease, pest tolerance, and agronomic traits during 2001. It is also the material used for the second evaluation of the presented study.

In 2000B, a cycle of the selection in S_1 lines in greenhouse. The genetic progress of complete resistance due to the two first selection cycles was considerable, with an important increase of the percentage of S_1 lines resistant to strains of the different lineages. In particular, three S_1 lines (equivalent of F_2 population) were obtained with complete resistance to all the strains that were tested (Valès, Rice Project Annual Report, 2000). The remaining seeds of the S_1 selected lines constituted the material that was used in another cycle of genetic recombination during 2001.

The second cycle of recurrent selection for blast resistance, pest tolerance, and other agronomic traits gave the opportunity to study the genetic progress for these characters. The trial was carried out in Santa Rosa experimental station, Villavicencio, Colombia during the first semester of 2001. The trial design was in Federer's blocks of 20 lines and 5 checks. Two rows of 5 m were sowed by material (19 kg/ha). The genetic gain was compared with that made in the first cycle of recurrent selection which had previously been tested using the same experimental design and check varieties. A *Panicum maximum* border was used to isolate the trial. The spreader rows consisted of Oryzica 1 were inoculated with dry leaves of plants infected with the rice blast strain Fanny 54 (10 g of dry leaves / m^2 of spreader).

Results and Discussion

These studies were done to quantify the genetic gain in yield and blast resistance that is being achieve using recurrent selection to enchance these traits. The statistical analysis used the data on the check varieties for avoiding the environmental differences between the evaluations.

The yields of the PCT-6 C_1 lines tended to be higher than those of the PCT-6 C_0 lines (Figure 2). This demonstrated the general progress for the due to the recurrent selection. The highest yield line was 6.750 t/ha in the PCT-6 C_0 and was 7.750 t/ha after the second cycle of recurrence selection (PCT-C_1). About 20% of the PCT-6 C_1 lines had yields of 3700kg or more compared with only 2% of the PCT-6 C_0 lines. Also it is a confirmation of the good potential of the PCT-6 population because these productions were obtained using the planting density equivalent to 19 kg of seeds / ha.

Panicle weight and number are two characteristics that can affect the yields of plants. The panicle weight (Figure 3) increased significantly in the PCT-6 C_1 lines as compared with the PCT-6 C_0 lines. The panicle number per plant was not significantly different between the two cycles of recurrent selection (Figure 4). This was evidence that a principal component of the yield increase was the panicle weight.

Although the correlation between disease progress and yield was relatively low, there were not lines highly susceptible to blast with high yields (Figure 5). Also, no progress for leaf blast resistance (Figure 6) or for neck blast resistance (Figure 7) was observed between the two cycles of recurrent selection. Perhaps it is due to the difficult interpretation of the data caused by the interaction for partial resistance between check varieties and strains. It was not the same strain for the both evaluations, and we know that the second strain was more aggressive.

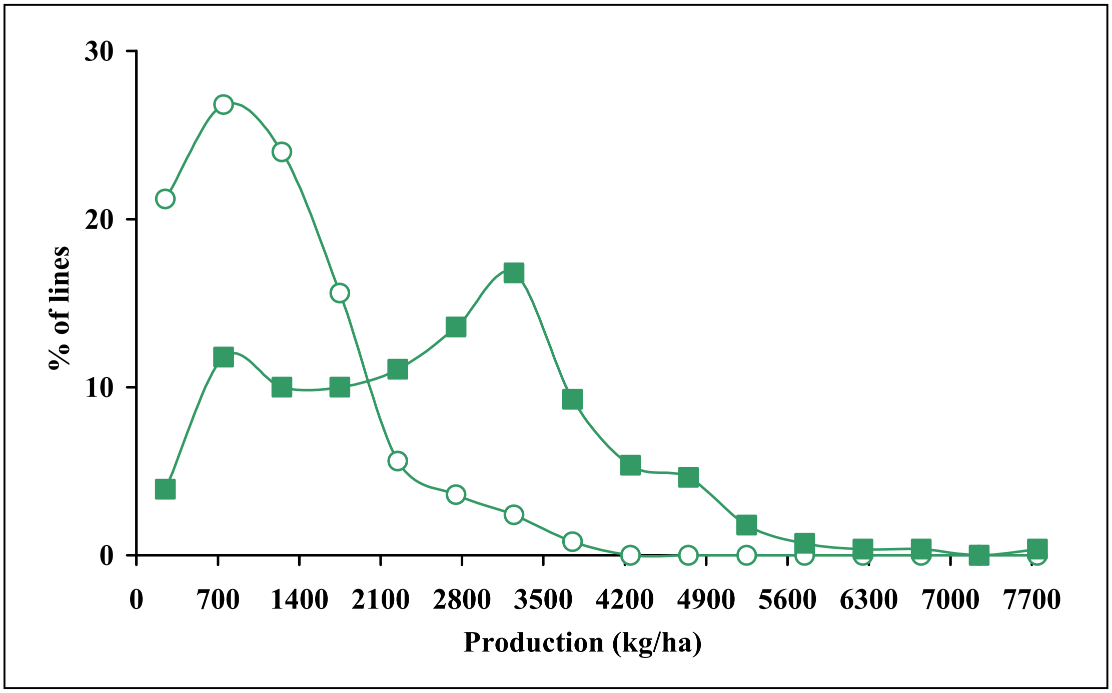


Figure 2. Yields in kg of lines from the recurrent selection (RS) population PCT-6. The circles represent lines from the PCT-6 C_0 population (after one cycle of RS) and the square represent lines from the PCT-6 C_1 population (after two cycles of RS)

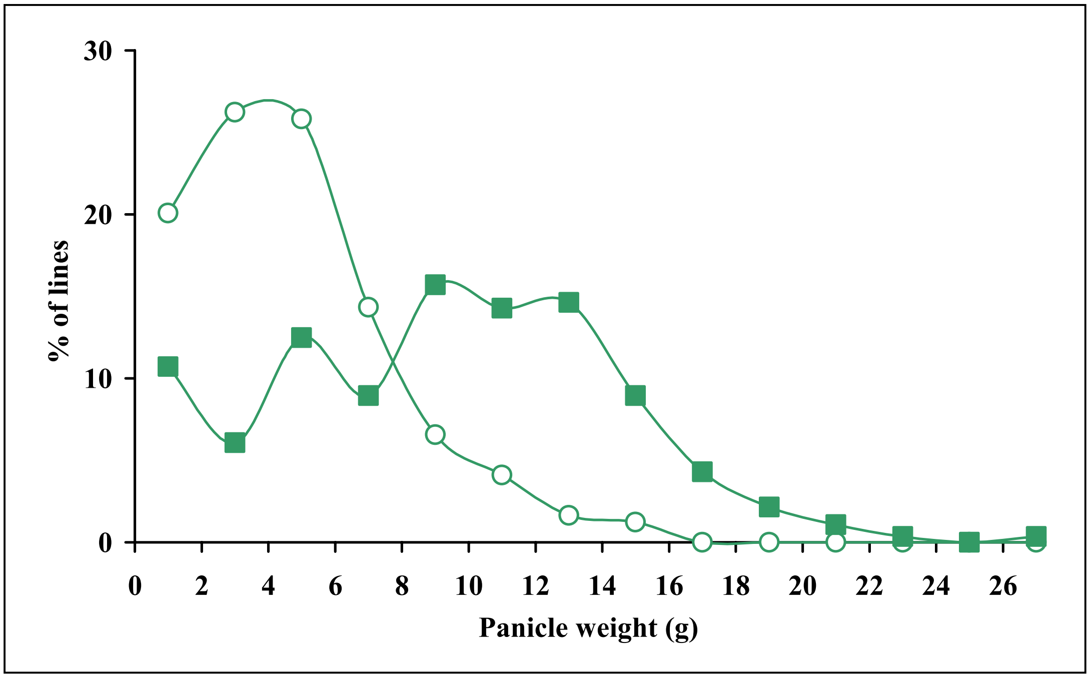


Figure 3. The panicle weight of lines from the recurrent selection (RS) population PCT-6. The circles represent lines from the PCT-6 C_0 population (after one cycle of RS) and the square represent lines from the PCT-6 C_1 population (after two cycles of RS)

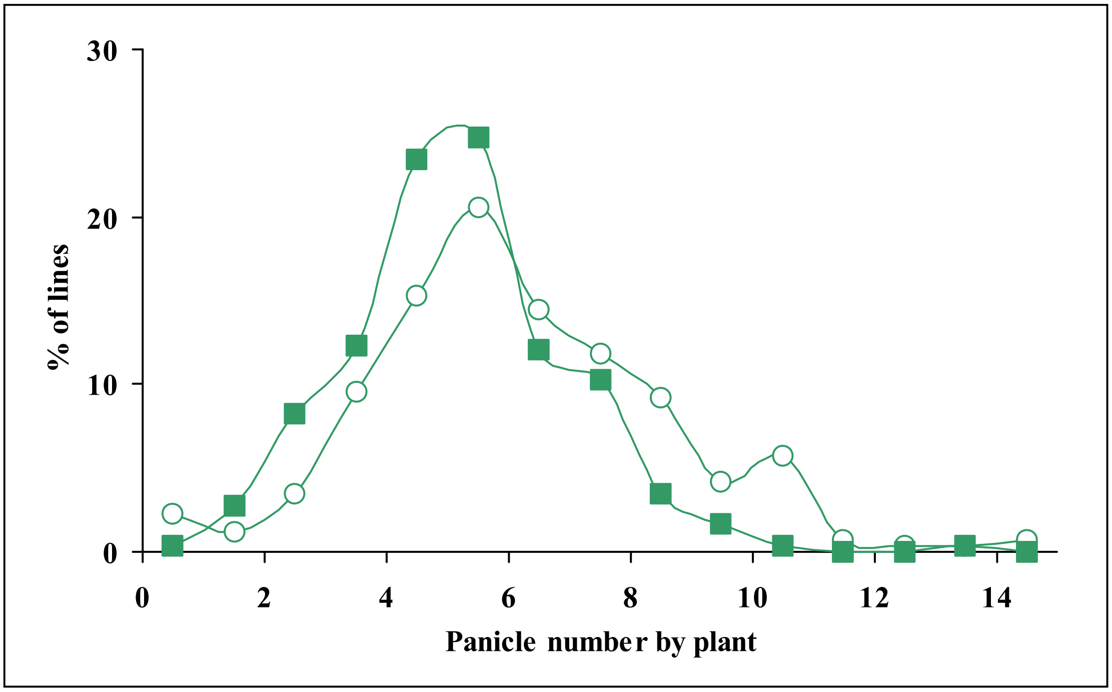


Figure 4. The panicle number per plant from the recurrent selection (RS) population PCT-6. The circles represent lines from the PCT-6 C_0 population (after one cycle of RS) and the square represent lines from the PCT-6 C_1 population (after two cycles of RS).

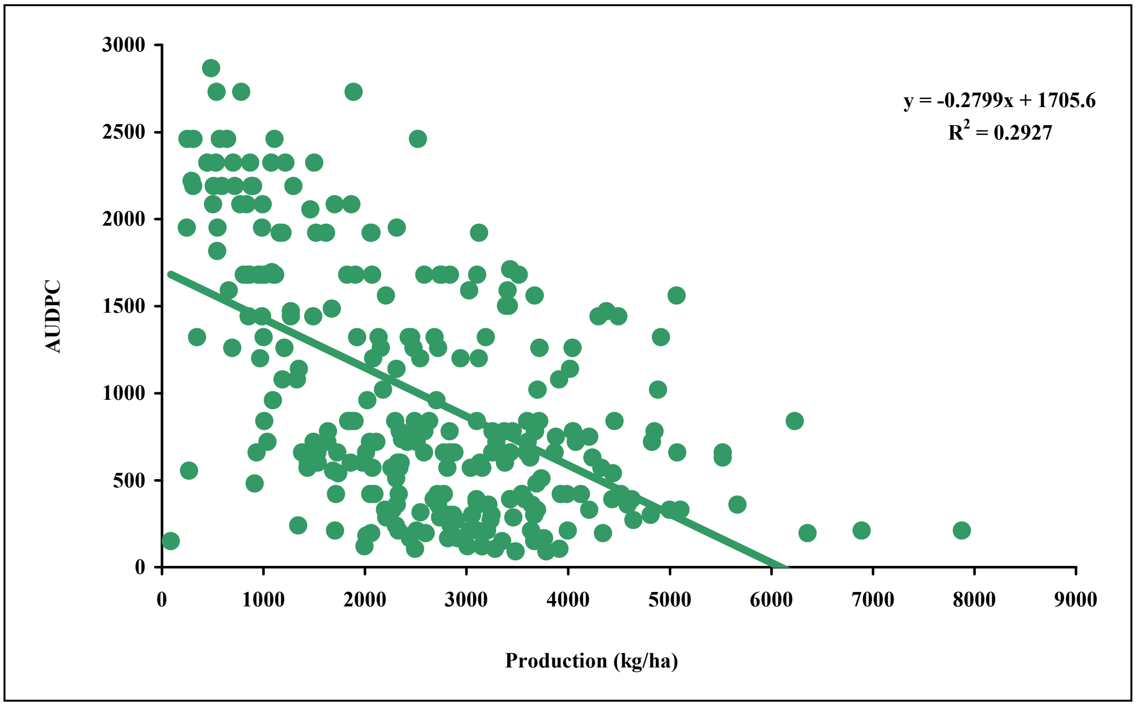


Figure 5. Relation between the Production and the Area Under the Progress Disease (Blast) Curve (UPDC) after the Cycle of Recurrent Selection.

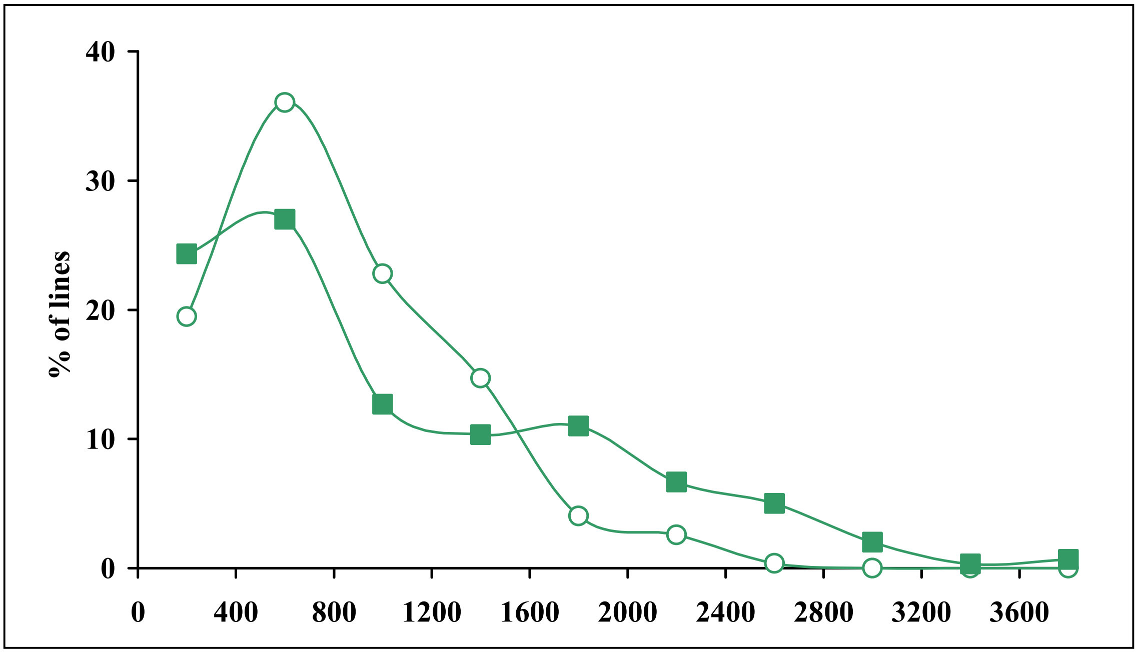


Figure 6. The reaction of the Area Under the Progress Disease (Blast) Curve (UPDC) of lines from the recurrent selection (RS) population PCT-6. The circles represent lines from the PCT-6 C_0 population (after one cycle of RS) and the square represent lines from the PCT-6 C_1 population (after two cycles of RS).

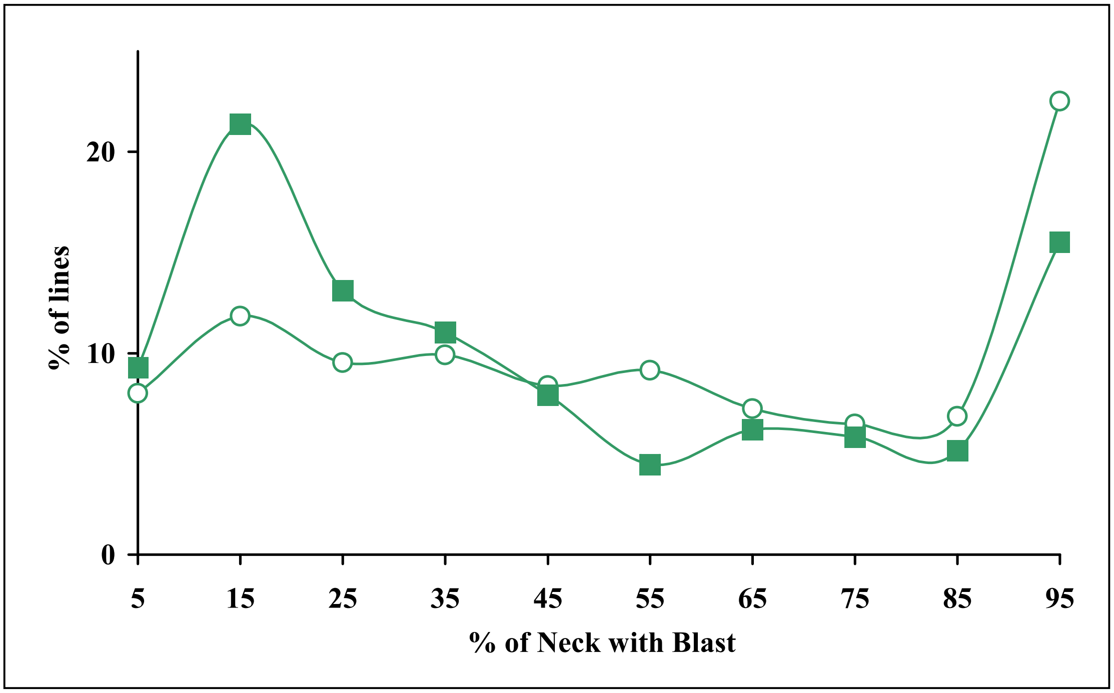


Figure 7. The percentage of lines from the recurrent selection (RS) population PCT-6 showing neck blast symptoms. The circles represent lines from the PCT-6 C_0 population (after one cycle of RS) and the square represent lines from the PCT-6 C_1 population (after two cycles of RS).

Perspectives

The more complete analysis of the data for evaluating of the progress obtained by a cycle of recurrent selection using our particular scheme are part Master Science thesis of the Ing. Joanna Dossmann.

The results demonstrate in a quantitative manner the types of yield gains that can be achieved through recurrent selection, this method is be used to enhance other recurrent populations, in particular those with narrow genetic bases.

We are collaborating with NARs to make this selection method widely available as a breeding tool.

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OUTPUT 2. CHARACTERIZING RICE PESTS AND THE GENETICS OF RESISTANCE

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

• Determining the Effect of *Tagosodes orizicolus* with different Levels of Virulence on Different Varieties of Rice

R. Meneses and M. Triana

Abstract

This study was conducted to determine the economic threshold for rice hoja blanca virus. This was highly dependent on the variety, and at the disease pressure level used in this study, there was significant yield losses to both susceptible and varieties with intermediate levels of resistance to RHBV. The highly resistant variety Fedearroz 2000 had less disease and the yield was not significantly different from the controls. Economic thresholds and levels of intervention depend on the disease pressure and the variety. This information is being used to refine integrated management strategy for RHBV.

Introduction

Rice hoja blanca virus (RHBV) is vectored by *T. orizicolus.* This planthopper also causes direct damage to rice. The interaction of the planthopper/virus and their effect on the yield of rice needs to be understood for each varieties in order to design integrated pest management strategies. In Colombia, the variety Fedearroz 50 is planted on more than 60% of the area and it has resistance to both RHBV and *T. orizicolus.* Since the release of this variety, the levels of RHBV have fallen sharply, and it is not currently a problem in most areas of Colombia. However, it is a variety with an intermediate level of resistance to RHBV and high disease pressure has the potential to cause losses in this variety. This study was undertaken to determine at what level of the planthopper/virus causes significant yield reductions in varieties with different levels of resistance. This information is needed in order to determine thresholds of economic damage to help develop guidelines on when to use control measures. This type of information is needed to help reduce the abuse of pesticides, which lowers the cost of production and reduces the contamination of the environment as well as assure that hoja blanca disease does not cause economic losses.

Materials and Methods

Each variety was planted at a density of 3 grams/line in plots of 1.95 X 1.50 m (12 lines of 1.5 meter in length) with 3 replicates per treatment. At 15 days after planting, each plot was covered with nylon mesh cages and were infested with adult *T. orizicolus*. After 5 days, the plots were treated with insecticide to kill the planthoppers. The varieties Fedearroz 50, Fedearroz 2000, Oryzica 1 and O. Caribe 8 were infested with 0 or 6 *T. orizicolus* with either low (less than 2%) or high (25% percent) of vectors as determined by ELISA. In order to determine the percentage of plants infected with RHBV, the total number of plants and the number of plant with symptoms of RHBV were counted. The evaluations for RHBV were made at 20 days after the infestation with the vectors. The yield of each treatment was calculated from the average of the harvest of the plots after the seed was dried to 14% humidity. To determine the significance of the treatments analysis of variance and tests of multiple comparisons were done.

Results and Discussion

Two different colonies (RHBV viruliferous and normal) were used in this experiment in order to determine if the insect alone was causing yield reductions at the level of 6 insects per plant. The colony maintained as the virus free colony does contain a low number of vectors, and there was some transmission especially in the susceptible variety. Still the two colonies had very different rates of transmission. In the intermediate resistant varieties of Fedearroz 50 the level of infection was about 5.5, and in Oryzica 1 the level was 5.4 and the susceptible variety O. Caribe 8, the rating was 8.6 (Figure 1). The difference between the virus treatment and the planthopper treatment were highly statistically significant. In comparison, Fedearroz 2000 had a rating of 1 and there were no significant differences between treatments. Although the planthopper treatment did not affect yields in a statistically significant manner for any of the varieties (Figure 2), there was a slight reduction in yield in three of the four varieties. Also there were no significant differences in the yield of Fedearroz 2000 in any of the treatments, but the yield was lower in the virus treatment. There were significant reductions in yields in the virus treatment for the other three varieties. For the susceptible variety O. Caribe 8, there was 80.7% yield reduction as compared with the control treatment. For the intermediate resistant varieties Fedearroz 50 and Oryzica 1, there were 43.2 and 37.0%, respectively, yield reductions as compared with their control treatments. The results of the study suggest that management practices may be able to be made on the basis of the category of resistance to RHBV. Both Oryzica 1 and Fedearroz 50 had nearly the same reaction at this level of disease pressure.

The information in this study is being used to refine integrate management strategies to prevent losses and pesticide abuse due to RHBV. Currently there are three classifications for resisitance to RHBV. The are susceptible, intermediate resistance and highly resistance. These preliminary results indicate that threshold levels for economic damage are similar for varieties with the same classification. This could allow for recommendation to be made by category and this would simplify the integrated management strategy for the complex of *T. orizicolus* and RHBV.

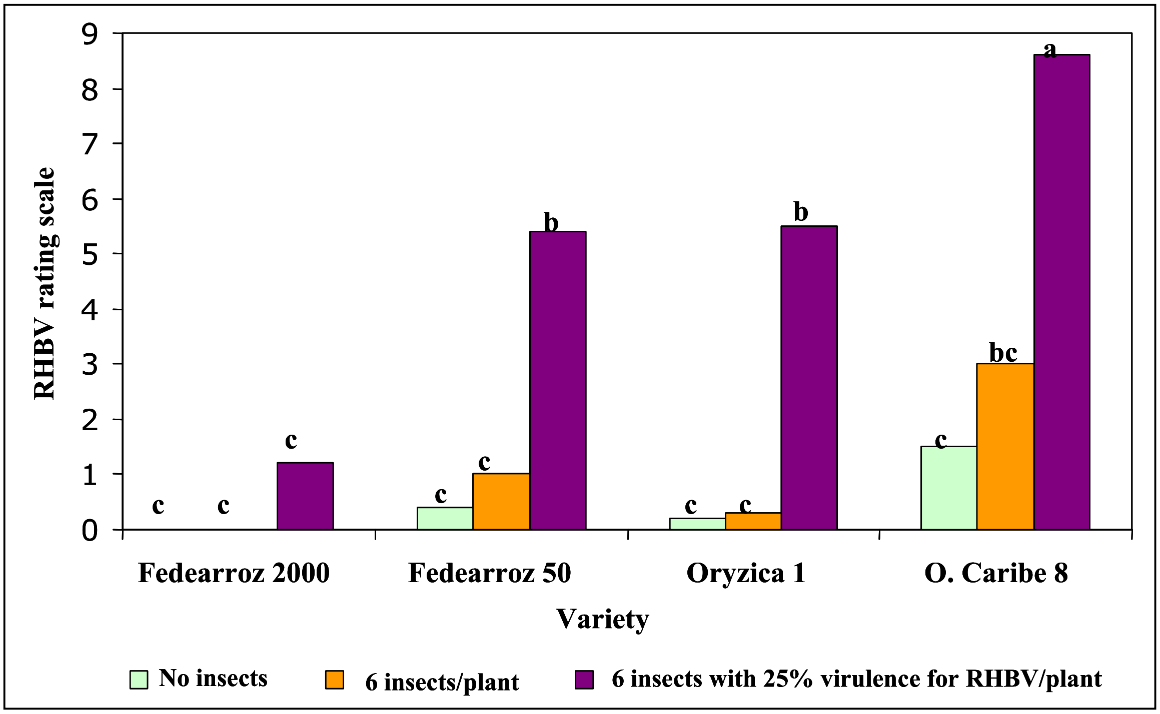

Figure 1. Comparing the incidence of RHBV using four rice varieties that were infested with *T. Orizicolus* that were either from a colony with a very low incidence of RHBV and another where 25% of the individuals were carriers of RHBV. The control groups were not infested with planthoppers.

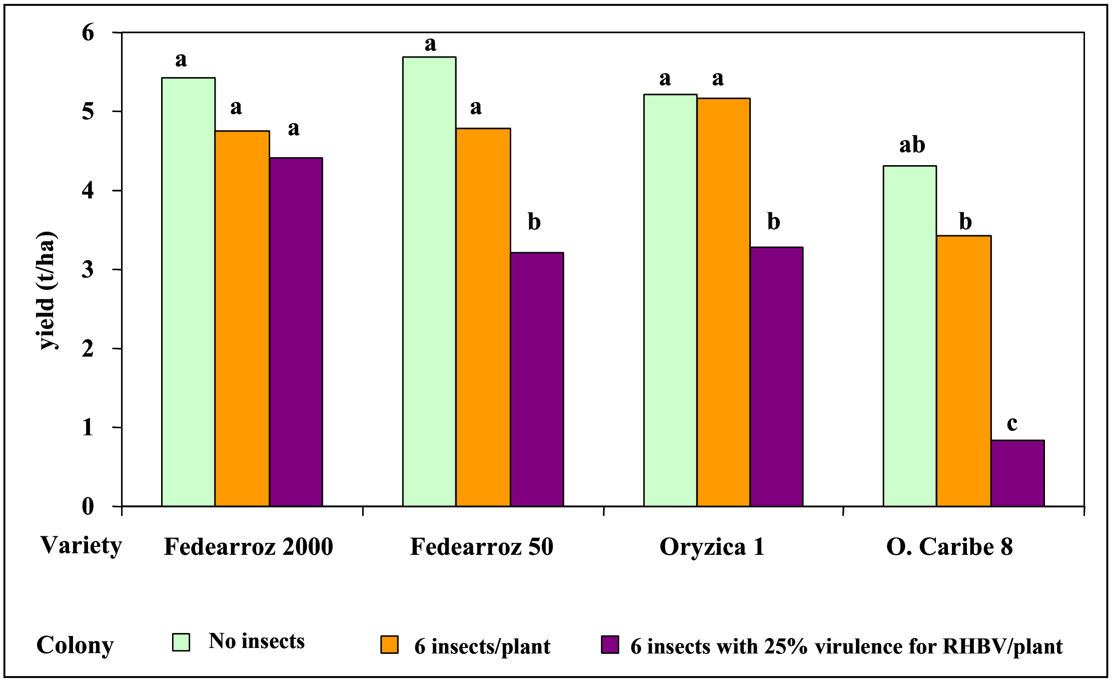

Figure 2. Comparing the yield of four rice varieties that were infested with *T. orizicolus* that were either from a colony with a very low incidence of RHBV and another where 25% of the individuals were carriers of RHBV. The control group were not infested with planthoppers

• A Comparison of Three Colonies of *Tagosodes orizicolus*

R. Meneses, M. Triana, L. Calvert

Abstract

Three different colonies of *T. orizicolus* were compared in field conditions for their ability to transmit RHBV. In field conditions, no differences were noted between the three colonies in respect to the transmission rates of RHBV.

Introduction

There are differences in the aggressiveness of different colonies of *T. orizicolus.* Over the last several evaluations, there is evidence that the CIAT Palmira colony has a better ability to transmit RHBV as compared with colonies for earlier years. This has led to results in which varieties with intermediate resistance to RHBV were so affected that they had a rating as if they were susceptible. Since these are the control varieties in the mass screening for resistance to RHBV, it becomes difficult to rate the materials. Although this has been corrected by lowering the number of vectors, a number of studies were initiated to better understand the interactions between the virus, vector and varieties.

Materials and Methods

Three colonies *T. orizicolus* were developed and maintained in cages in greenhouses. The planthoppers were collected from Valle del Cauca to develop the colony Ginebra, and from the Atlantic coast to develop the colony Fundación. The third colony CIAT Palmira was developed three years ago using insects from a previous colony as well as planthoppers collected in Tolima and Valle del Cauca. The RHBV highly resistant rice variety Fedearroz 2000, the intermediate resistant varieties Fedearroz 50 and Colombia 1 as well as the susceptible rice line WC 366 were used in this study. The field experimental design consisted of four repetitions of each treatment in 1 x 1 meter plots with 7 rows and these plots were covered with a fine mesh cloth. The plots were infested using approximate one insect per plant at 15 days after planting, and the plots were fumigate 5 days after the infestation. Each plant in interior three rows was rated for presence or absence of RHBV symptoms at 30 days after the infestation. The five interior rows were harvested, the seeds dried and the yield was determined. The results were analyzed using an analysis of variance (SAS program).

Results and Discussion

At the evaluation at 30 days after infestation, the incidence of RHBV was significantly different between Fedearroz 2000, the intermediate resistant varieties of Colombia 1 and Fedearroz 50 and the susceptible WC 366 (Figure 3). The were no significant differences between the level of infection with RHBV between the colonies CIAT Palmira, Ginebra and Fundación. In addition, there were no tendencies for one variety to vector the virus under field conditions better than the others. For field testing, it appears that the number of vectors and the percentage of RHBV positive insects are the key factors in controlling the disease pressure.

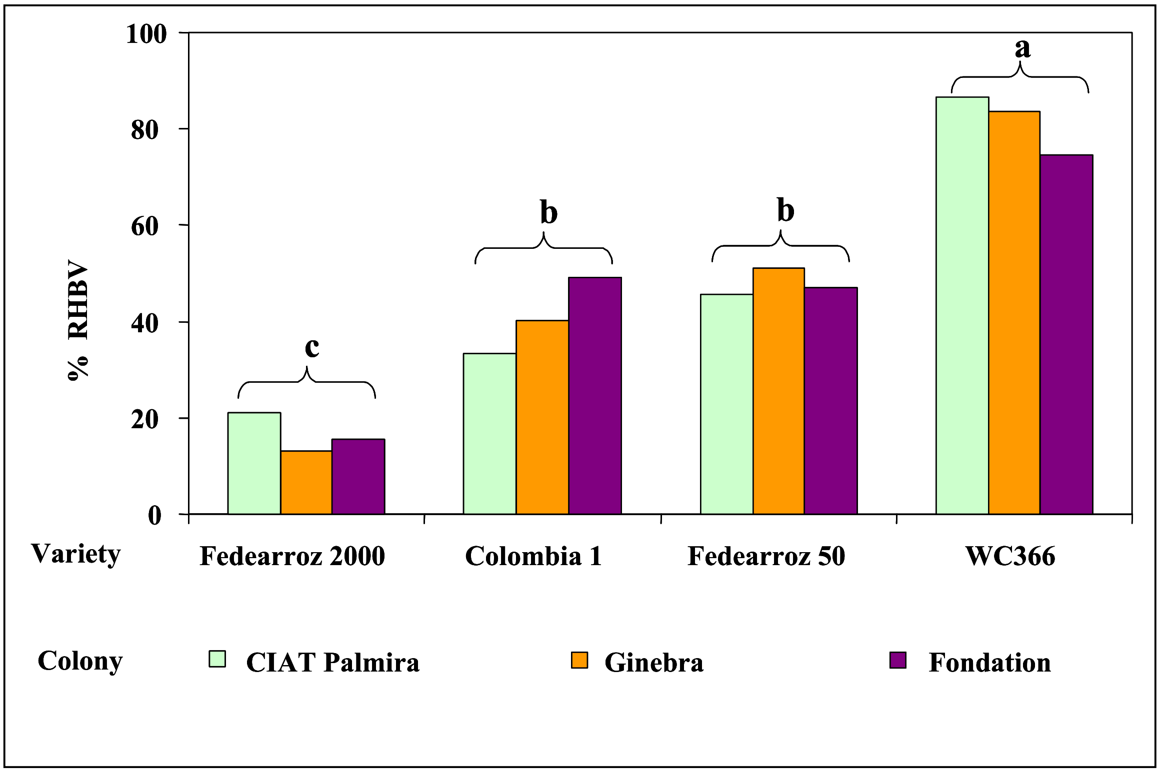

Figure 3. A field test comparing three colonies of *T. orizicolus* and their ability to transmit RHBV to four rice lines

Understanding the Interaction between RHBV, *T. orizicolus* and Rice Varieties

L. Calvert, R. Sedano, I. Lozano, M. Cuervo, R. Meneses, M. Triana, J. Silva, M. Duque.

Abstract

A study was made of two colonies of *T. orizicolus* and their ability to transmit RHBV. After five days on individual plants, each insect was test for RHBV and the virus titer was quantified. There was a correlation between the amount of virus in the insects and their ability to transmit RHBV. The older colony, which was founded three years ago, had a significant higher percentage of individuals with high virus titers as compared with the colony that was established less than one year ago. This is probably occurring because of the selection pressure of the virus. The insects that cannot tolerate high virus are eliminated as the colony is maintained over many generations. This could explain why this colony appears to be more aggressive in respect to the transmission of RHBV.

Introduction

The virus, vector and host are all important components in the dynamics interactions of RHBV epidemics. There are many reports concerning the dosage effect, which states that increasing the number of vectors per plant causes an increase in the rate of transmission. However, in recent years, it is clear that not all sources of virulent *T. orizicolus* are equal in their ability to transmit RHBV, and there is observational evidence that this is not just a function of the percentage of vectors in a colony. To complement the field study that compared three colonies of *T. orizicolus*, a controlled study using single individuals per plant was undertaken. The idea was to determine if the two colonies were similar in their ability to transmit RHBV and to better understand the components, which influence transmission.

Materials and Methods

Two colonies of *T. orizicolus* were used in this study are the colony CIAT Palmira and the colony CIAT Caribe which were described in the section 'A comparison of three colonies of *T. orizicolus*'. The colonies were maintained on Bluebonnet 50, and insects from the original colonies were tested using ELISA for the presence of RHBV. The three sources of rice used in this study were Fedearroz 2000, which is highly resistant, Fedearroz 50, which is intermediate resistance and WC 366, which is highly susceptible to RHBV. The rice seedlings were covered using acetate tubes and infested with one *T. orizicolus* per plant at 15 days after planting. After 5 days, 100 insects for each variety were collected and tested for RHBV. The ELISA values less than 0.03 were considered negative, 0.03 to 0.1 were rated as low virus titer, 0.11- to 0.3 as medium virus, and above 0.3 as high virus titer. Thirty days after the insects were removed, the plants were evaluated for RHBV. For the analysis of the virus levels in the original colony Chi square tests were done. To understand the interactions an analysis of CATMOD (SAS package) for the factors of level of virus in the insects, source of the insects (colony), and the rice variety were done.

Results

The Status of Two Colonies of *T. orizicolus* for Level of RHBV. When insects were collected directly from the colonies, approximately 18% of the individuals were negative for RHBV. In the range of low and medium titer, there were a greater percentage of individuals positive for RHBV in the colony of CIAT Caribe than the colony CIAT Palmira (Figure 4). This was reversed for group with high virus titers, where 49% of the individuals in the colony CIAT Palmira had high virus titers compared with 14.5% of the individuals in the colony CIAT Caribe. When comparing each group using a Chi square analysis, except for the number of negatives, the differences between the percentage of planthoppers in each grouping (low, medium, and high virus titer) were statistically significant.

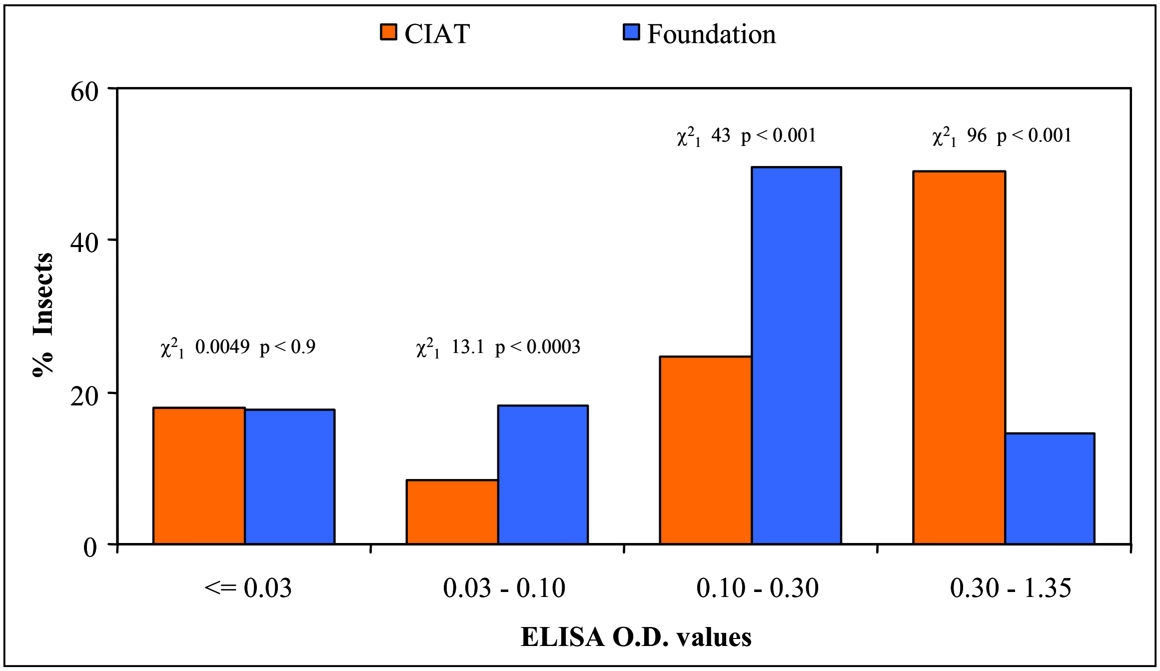

Figure 4. The distribution of individuals as a function of virus load as determined by ELISA O.D. Values for the two colonies of *T. orizicolus*

The Interaction of Virus Titer in the Insect and Varieties

The insects that were positive for RHBV from both colonies had homogenous distribution on each of the rice varieties; χ_2^2 0.204 P=0.9. The efficiency of transmission increased with as the titer in the insects increased (Figure 5). When grouping all three varieties, the insects with high titers of RHBV (0.3) had a transmission rate of 59.7 %, insects with medium titers (0.1-0.3) had a transmission rate of 43.7%, insects with low titers (0.03-0.1) had a transmission rate of 18.6%, and those that were rated negative had a transmission rate of 9.0%. The transmission from insects in which RHBV could not be detected was unexpected. Nevertheless, there was a strong correlation between virus titer and the rate at which the vectors were able to transmit RHBV.

There were notable differences in the incidence of virus transmission in the three varieties. In the most susceptible variety, the insects with medium and high titers were nearly equal as vectors, while in the intermediate resistant variety Fedearroz 50, insects with the high titers were significantly better able to transmit RHBV than those with medium or low virus titers. For the most resistant variety Fedearroz 2000. The medium and high titer insects were equal in their ability to transmit RHBV albeit at levels much lower rates than the other varieties. This suggest that the virus dosage was most important in the breakdown of resistance for the varieties with an intermediate level of RHBV resistance.

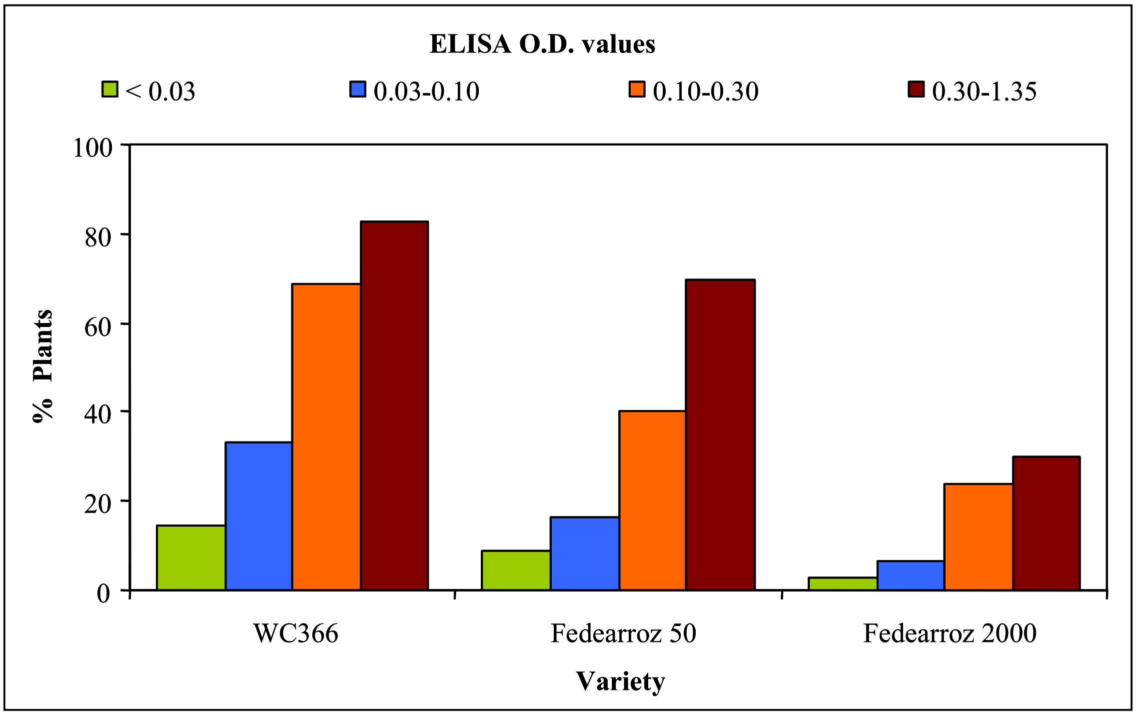

Figure 5. The Interaction of Virus Titer in the Vector (ELISA O.D. Values), Varieties and the Percentage of Plants the Became Infected with RHBV

The Colony and its Influence in Virus Transmission

For the susceptible variety WC-366 and the highly resistant variety Fedearroz 2000, the source of the insect did not matter. For the variety with intermediate resistance Fedearroz 50, the colony was important. The CIAT Palmira colony caused a higher level of infect than the colony Caribe (Figure 6) and using the Chi square analysis this was significant difference: χ_1^2 10.57 P=0.001.

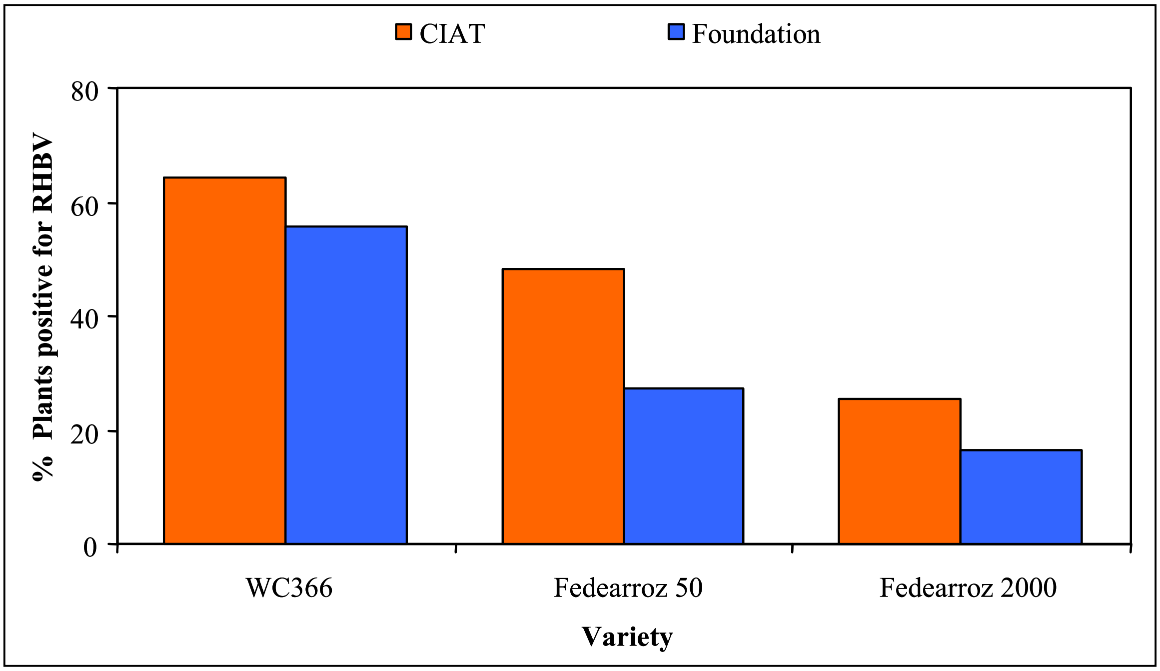

Figure 6. The interaction of the colony and the varieties. The only interaction where the colony caused a significant effect was with intermediate RHBV resistant variety Fedearroz 50

Predicting the Probability of a Plant becoming Infected with RHBV

From the analysis of traits, the probability [p(a)], that a rice plant would become infected with RHBV when infested with a single insect, depends on the following principal factors: the insect colony (C); χ_1^2 4.38 P=0.036, the rice line (M); χ_2^2 32.85 P=0.0001 and the level of titer in the insect (S); χ_1^2 20.49 P=0.0001. In this model, the interactions between the factors did not contribute in a manner that was statistically significantly (p>0.13). Using the low titer group as the baseline, the medium titer group caused an increase of 22.5% and the high titer group caused an increase of 41.3% in plants that were RHBV positive. Also, insects from the colony CIAT Palmira contributed to an 11.2% increase of RHBV positive plants as compared to insects from the colony Caribe. The variety was also very important and the susceptible line WC 366 has 41.3% more incidence of RHBV and Fedearroz 50 22.5% more incidence of RHBV than Fedearroz 2000. The incidence of transmission in each principal factor can be calculated as a probability factor.

An additive model was tested to see if it would be able to predict the incidence of RHBV in this experiment. The principal factors of colony (C), rice line (M), and virus titer (S) were summed to give a predicted value for the percentage of transmission (p9a)) of RHBV: p (a)=β+C+M+S; where β is an intrinsic value. The predictions of the model had a correlation with the experimental results of R^2 =0.808 (Figure 7).

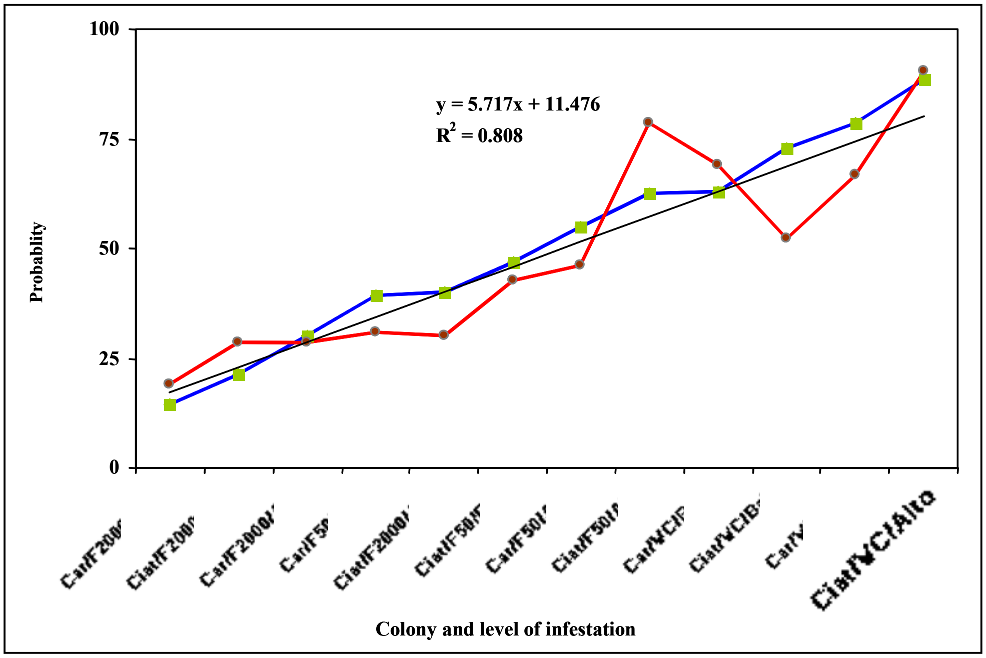

Figure 7. The correlation between the predicted level of infection with RHBV (probability) and the experimental values. The squares are the predicted values, the dots are the observed values. The treatments were sorted by the predicted probability of infections. The factors are colony ciat or car, the rice line Fedearroz 2000 (F2000), Fedearroz 50 (F50), WC 366 (WC), and high (alto) or low (bajo) level of virus titer in the vectors

Discussion

In order to understand the interactions between the host, vector and virus, we need to know the importance of the different components. This experiment is an attempt to quantify these components. The two principal factors were the variety and the titer of virus in the individual insects. The source of the insects was also important. This was also an attempt to quantify the dosage effect. There are several reports that increasing the number of vectors, increases the rate of transmission of RHBV. This study indicates that the titer within an individual also contributes to the dosage effect. A model was developed that predicted the rate of transmission base on the principal components. In order to increase our confidence in this model, additional experiments need to done.

This study has several different uses. First it is being used to better understand our colonies in order that we can better predict what number of insects needs to be used in the field screening trials for RHBV. This study as increased our understanding the variations in the colonies of *T. orizicolus* and now needs to be incorporated into our screening methodology.

The model developed will also be used in the effort to predict the probability of outbreaks of RHBV. This information can be used with the information being generated the threshold levels to develop a more accurate list of recommendations for interventions to prevent losses and pesticide abuse due to RHBV. The third use of this study will be in the development of molecular markers for resistance to RHBV. Resistance is not black and white. It is an interaction of the vector, virus and the rice line. Individual plants from even the most resistant commercial variety can become infected with RHBV. The key to developing the markers will be the biological tests. A predictive model indicating the probability that an individual plant will become infected is needed in this effort.

Acknowledgements

In alphabetical order, we thank the rice entomology group that maintains the colonies, Efrén Córdoba, Mauricio Morales y Rodrigo Moran. We also wish to express our appreciation for the collaboration of Natalia Villarreal and Ana Cecilia Velasco.

• Preferences for Oviposting Eggs on Ten Rice Lines using Force Feeding

M. Triana, R. Meneses y L. Calvert

Abstract

The planthopper *T. orizicolus* is a significant pest on rice, and it is the vector of RHBV. In order to better understand the components of resistance to both the insect and virus, preferences for oviposting eggs and the longevity of females were determined. There was a strong correlation between longevity and the number of eggs oviposted. There was a tendency for the oviposition of eggs to be associated with the varieties with resistance to RHBV. Of the four varieties that have resistance to RHBV, only Colombia 1 was a preferred host to the insect. All six of the RHBV susceptible rice lines were preferred host for the insect. The least preferred host was Fedearroz 50, which is also intermediate in resistance to RHBV. This trait could contribute to field resistance to RHBV by help to maintain lower populations of the vector.

Introduction

In order to understand the principal factors of resistance to *Tagosodes orizicolus*, we have made several studies concerning longevity, preference studies, life cycles, and other factors. Ten different rice lines have been selected as core varieties, and these are being used as parents in specific crosses to better understand the genetics and to develop molecular for the components of resistant to *T. orizicolus* and RHBV. This study focuses on the influence of the rice line on the oviposting of eggs and the longevity of the planthoppers.

Materials and Methods

The varieties used in this study were Cica 8, Oryzica Llanos 5, Colombia 1, Oryzica 1, Fedearroz 50, IR 8, Bluebonnet 50, WC 352 (CT10871-1-CA-1-M), WC366 (IR65598-27-3-1) and Fedearroz 2000. Ten plants of each variety were placed individually into acetate tube 25 days after planting. A pair of recently emerged adults was placed on each plant. The pair planthopper were transferred to new plants of the same variety every eight days until the death of the female. The plants were examined using a microscope and the number of eggs oviposted were counted. The experiment was replicated three times using a complete randomized design. The variables of the number of oviposited eggs and longevity were analyzed by ANOVA (SAS program).

Results

For most of the varieties in this study, there were not significant differences for either oviposting of eggs or longevity. Colombia 1, WC 352, Bluebonnet 50, IR 8, WC 366, O. Llanos 5 were in the susceptible group for both traits (Table 1). There was a significant difference between Cica 8 and the Colombia 1 and WC 352 for the number of eggs but not for longevity. The varieties Oryzica 1 and Fedearroz 2000 had a highly significant effect

Table 1. Preference in oviposting of eggs and the life expectancy of *T. orizicolus* females force fed on ten rice lines.

Varieties	Average number of eggs per female	Average life expectancy of females in days
Colombia 1	333.7 a[1]	25.8 a
WC 352	327.5 a	27.9 a
Bluebonnet 50	318.4 ab	25.6 ab
IR 8	294.4 ab	24.2 ab
WC 366	287.4 ab	22.6 ab
Oryzica llanos 5	241.7 ab	22.4 ab
Cica 8	217.8 b	24.4 ab
Oryzica 1	169.1 c	22.9 ab
Fedearroz 2000	129.6 c	20.7 b
Fedearroz 50	38.3 d	14.2 c

[1] The different letters indicate that the results were significant at the 0.05 level (Duncan, alpha =0.05).

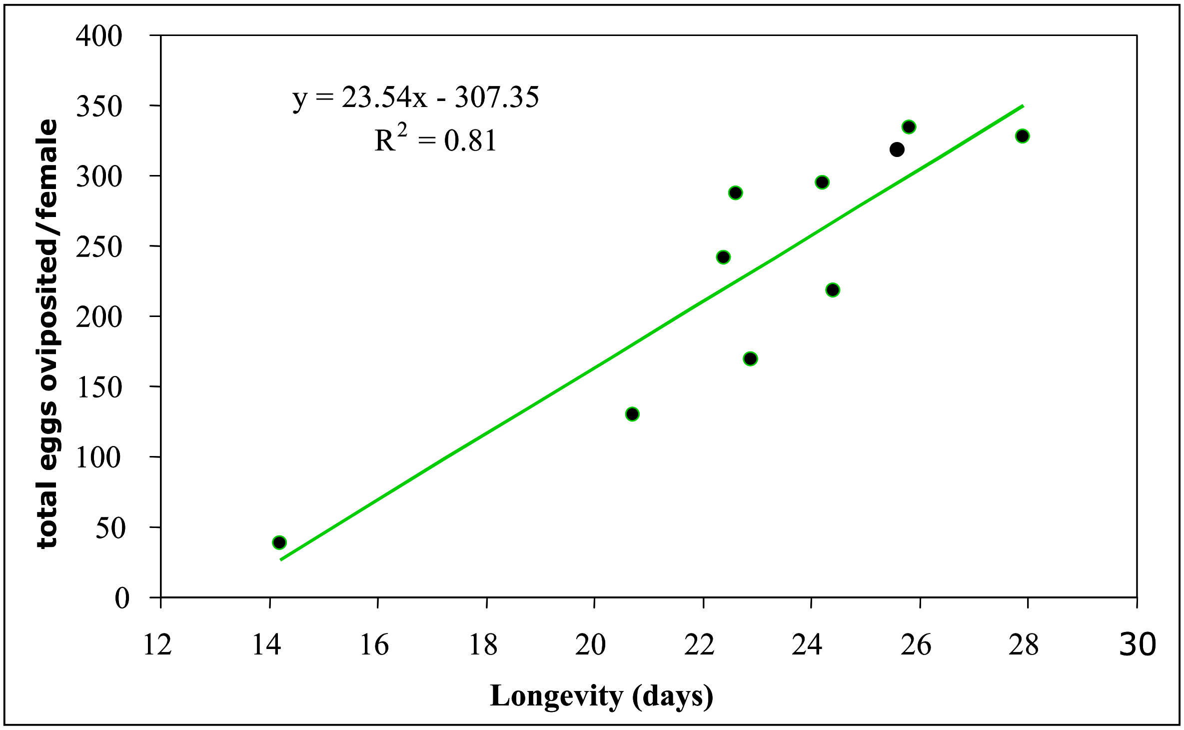

Figure 8. The Correlation between the Number of Eggs Oviposted and the Longevity of the Female *T. orizicolus*

on the number of eggs and were more resistant that the susceptible group. There was less variation for longevity with Oryzica 1 being in the susceptible group and Fedearroz 2000 only was different from Colombia 1 and WC 352. The variety Fedearroz 50 had the least number of oviposted eggs with an average of 38.3 and the female longevity was averaged 14.2 days. The longevity of the planthoppers on Fedearroz 50 is approximately 10 days less and the number of eggs was less than 20% of the average for the susceptible varieties. There is a relation between the number of eggs oviposted and the longevity of the females (Figure 8). Using regression analysis the correlation factor was $R^2 = 0.81$.

Discussion

In the group of susceptible varieties for these traits, which are components of resistance to planthoppers, were Colombia 1 and O. llanos 5. This reaffirms other studies that indicate that some components of resistance to RHBV are distinct from the resistance components of *T. orizicolus*. Oryzica 1 and Fedearroz 50 have intermediate resistance to RHBV, and Fedearroz 2000 has a high level of resistance. All three varieties had a lower level of ovipositing of eggs as compared with the other varieties. This trait would contribute to field resistance to both the virus and the planthopper by contributing to reduced populations. These results are consistent with previous studies including our report last year, and this gives us the confidence that is needed to include this trait in the molecular marker studies. The results are also important for the implications over the effect of *T. orizicolus* populations in areas where these varieties are widely grown. Especially in the case of Fedearroz 50, vigilance is needed to see if new biotypes adapted to this cultivar arise.

- ## Evaluation of the Effect of RHBV on the Yield of Ten Rice Lines

L. Calvert, M. Triana, L. Reyes (Fedearroz), and R. Meneses

Abstract

It has been said that the RHBV screening method does not produce consistent results. This field experiment was a center of focus, because it appeared that even the intermediate resistant varieties were susceptible to RHBV. A careful analysis of this disease rating at the different dates show that even under very high disease pressure intermediate varieties were easy to distinguish from susceptible varieties especially in the yield component. Further, this level of disease pressure highlights the excellent progress that has been made in developing new varieties with resistance to RHBV. Fedearroz 2000 was clearly superior to even the primary source of resistance Colombia 1. Fedearroz 2000 was screened and selected as an advance line under high disease pressure. This affirms that a two step screening process in which advance lines are subjected to high disease pressure is an appropriate screening methodology and that the results have exceed our expectations by producing varieties that are better than the parental sources.

Introduction

Last year we reported on field trials of ten varieties of rice infested at four different dates with RHBV viruliferous vectors. This update includes the information on the yield of those rice lines.

Methods and Materials

The varieties tested were CICA 8, Oryzica Llanos 5, Colombia 1, Oryzica 1, Fedearroz 50, IR 8, Bluebonnet 50, CT10871-1-CA-1-M, IR65598-27-3-1 and Fedearroz 2000. Three replications for each treatment were planted in a randomized block with plots of 2 m^2. The materials were infested with *T. orizicolus* at 7, 14, 21 y 28 after plant at the level of 1 insect/plant using a colony in which 70% of the planthoppers were virulence. The controls were not infested with *T. orizicolus.* At 30 days after the infestation, the plots were visually rated using a scale of 1 to 9 (IRRI). The analysis was an ANOVA using the yield data from the four infestation dates and the control plots which were not infested with vectors of RHBV.

Results

Colombia 1 has often been used as a source of resistance to RHBV, although it is not immune and can be infected especially during the seedling stage. Increasing the dosage as measured in the number of vectors used in the infestation can increase the incidence of RHBV. Using the CIAT Palmira colony that was known to transmit RHBV very efficiently, the rice lines in this trial were subjected to very high disease pressure. Nearly all of the varieties had a reaction as if they were susceptible to RHBV at all ages tested. Therefore, the yield and incidence data are presented as global values with all four infestation dates averaged together. The rice lines WC 352, IR-8, WC366, Bluebonnet 50, Cica 8 and O. Llanos 5 form a group of lines that are susceptible to RHBV, and the disease caused very high yield losses (Table 2). The intermediate resistant varieties of Colombia 1, Oryzica 1, and Fedearroz 50 sustained substantial yield losses, but these varieties showed more resistance when the infestation is later. For example, Oryzica 1 the disease ratings were 9.0 when infested at seven days after plant and the yield was 1.8 t/ha, and this compares with a disease rating of 7.7 and a yield of 4.6 t/ha when it is infested at 28 days after planting. The only variety, which had a high level of resistance to RHBV, was Fedearroz 2000. It had an intermediate reaction of 5.6 and 5.4 at 7 and 14 days, respectively, and a resistant score of 3.2 and 2.6 at 21 and 28 days after planting, respectively. The only treatment, which caused yield losses, was the 7 days after planting infestation and the yield was 5.8 t/ha. In the 14, 21, and 28 days infestations the yields were 8.6, 7.9 and 9.0 respectively as this is compared with a yield of 10.1 tons in the control for Fedearroz 2000 in which the yields averaged 10.1 t/ha.

Table 2. The level of infection of RHBV and the yield of ten rice lines infected 7, 14, 21 and 28 days after planting

	Infestation with 1 insect/ planta		**Control No infestation**	
Variety	**RHBV**	**t/ha**	**RVHV**	**t/ha**
WC 352	9.0 a[1]	0.7 g	0.8 e	6.0 d
IR 8	9.0 a	0.8 g	0.7 e	8.0 bc
WC 366	9.0 a	1.1 g	0.8 e	6.1 d
Bluebonnet 50	8.6 ab	1.4 g	0.9 e	8.1 bc
Cica 8	8.5 ab	1.6 g	0.8 e	7.6 c
O.Llanos 5	9.0 a	1.6 g	0.7 e	9.0 b
Colombia 1	8.5 ab	3.1 f	0.4 ef	9.0 b
Oryzica 1	8.3 bc	3.8 ef	0.5 ef	9.0 b
Fedearroz 50	7.9 c	4.3 e	0.4 ef	8.4 bc
Fedearroz 2000	4.2 d	7.8 c	0.0 f	10.1 a

[1] The different letters indicate that the results were significant at the 0.05 level (Duncan, alpha =0.05). The analysis of the levels of RHBV and the yield were independent.

Discussion

This confirms that rice lines with resistance to RHBV are less affected when the infestation occurs later in the season. Also the earlier the infection, the greater the yield loss. The high level of disease pressure in this experiment highlighted the differences between the resistant varieties as well as the susceptible varieties. Fedearroz 2000 was much more resistant than Colombia 1 the traditional source of resistance to RHBV. Fedearroz 2000 should be consider as a new source of RHBV and be used as a parent for this trait. It is also been selected as the most important parent for the molecular marker study to identify the genes involved in RHBV resistance.

- **Understanding the Mechanism in Transgenic Rice with Resistance to RHBV**

A. Garavito and L. Calvert

Introduction

The transgenic rice lines, that contain the nucleoprotein (N) gene, express low levels of the RNA transcript and the expression of the N protein has never been detected. This suggests that post-transcription gene silencing (PTGS) is a mechanism of the RHBV resistance (Lentini et al., 2002). Post-transcriptional gene silencing has been demonstrated in other transgenic plants (Vaucheret et al, 2001), including rice. It has also been demonstrated to be part of the natural defense mechanism in plants (Voinnet, 2001). Small RNAs of 21-25 nucleotides have been associated with PTGS and as a natural defense to plant viruses (Hamilton and Baulcombe, 1999). This study was undertaken to better understand the mechanism of the transgenic resistance to RHBV.

Materials and Methods

Biological Material

The control in all experiments were *Oryza sativa* L. *indica* cv Cica 8 which is susceptible to RHBV. In these experiment four transgenic lines containing the N-gene of RHBV and derived from Cica 8 were used. The transgenic lines A3 49 60 12 3 3 57-5 (57-5) and A3 49 60 19 12 6 (12-6) were resistant to RHBV, while the transgenic lines A3 49 60 12 3 1 31 4 (31-4) and A3 49 60 7 26 3 (26-3) were susceptible to RHBV. To inoculate the plants with RHBV, they were infested with virulent 2º or 3º instar virulent nymphs of the planthopper *T. orizicolus* at 10 to 15 days after planting. After five days the plants were fumigated.

The Extraction of RNA

Total RNA was extracted from the leaves using the Qiagen RNA easy protocol. The RNA was separated by size using denaturing formaldehyde gel electrophoresis and visualized after straining with ethidium bromide. In some experiments, the total RNA was treated with RQ Dnase.

Small molecular weight RNAs were purified using lithium precipitation or anionic column chromatography using the Qiagen RNA/DNA kit. The RNAs were separated by size using 15% polyacrylamide gel electrophoresis containing urea 7M in 0.5X TBE and visualized after straining with ethidium bromide. After the gel electrophoresis, the products were transferred to nylon membranes. The cDNA clone of the N-gene of RHBV (pVR3) was labeled [α-^{32}P] dATP and used as a hybridization probe to determine which products were homologous with the N-gene. The results were visualized using X-omat film.

RT-PCR and nested PCR

The protocols and primers for rt-PCR and nested PCR were performed as describe in the Lentini et al., (2002). In addition the primer 5’-ATCATCAAGCAAGAGTGGTTTCTGA

(RHB3-11) and 5'-GAAAAATCTTGTAAATATGTAAATACTC (RHB3-1375) were used for some of the reactions.

Serological Testing for RHBV.

The level of RHBV in the plants was determined using DAS-ELISA. The vegetative materials were diluted 1/100 (w/v) and the antiserum and conjugate were used at dilutions of 1/3000.

Results and Discussion

Evaluation of the Expression of the Transgenic N-gene

To determine if there was expression of the RNA transcript of the transgene, plants of different ages were tested. Using Northern blot analyses or rt-PRC, it was not possible to detect the transcription of the N-gene. Using a nested PCR to amplify the products of the rt-PCR, RNA transcripts of the N-gene were detected in most of the transgenic plants.

Replication of RHBV RNA3 in Inoculated Plants

To determine if there were differences in the accumulation of RHBV RNA 3, non-transformed susceptible rice, N-gene transgenic susceptible and resistant lines were tested at 10 days after inoculation. Using Northern blot analyses, the RHBV RNA 3 was detected in the non-transgenic control and the susceptible transgenic plants, but it was not detected in the transgenic lines with resistance to RHBV. This demonstrates that the transgenic resistance impedes the accumulation of RHBV RNA 3.

RNAs of Low Molecular Weight

Small molecular weight RNAs of 20-25 nucleotides have been detected in transgenic plants in which post-transcription gene silencing is a mechanism of resistance. Using plants of 45 days after inoculation, the only low molecular weight RNAs that were specific for the N-gene were 60-70 nucleotides, and these were found only in the susceptible plants. Similar results were obtained when low molecular weight RNAs were extracted from plants 10 and 20 days after inoculation. No RNAs of 20-25 nucleotides could be detected in any of the plants but the 60-70 could be found in the susceptible plants although they were in apparently lower quantities than the plants from the plants that were tested at 45 days after inoculation.

No small RNAs of 21 a 25 nucleotides were detected, and the extractions were made 10, 20 and 45 days after the inoculations. Small RNAs of 60-70 nucleotides were detected in the susceptible transgenic and non-transgenic plants using the RNA N-gene as a hybridization probe (Figure 9). The function of these small RNAs is not known. Additional experiments are being made to determine if the small RNAs associated with the mechanism of gene silencing are present and to identify the small RNAs found in RHBV infected plants.

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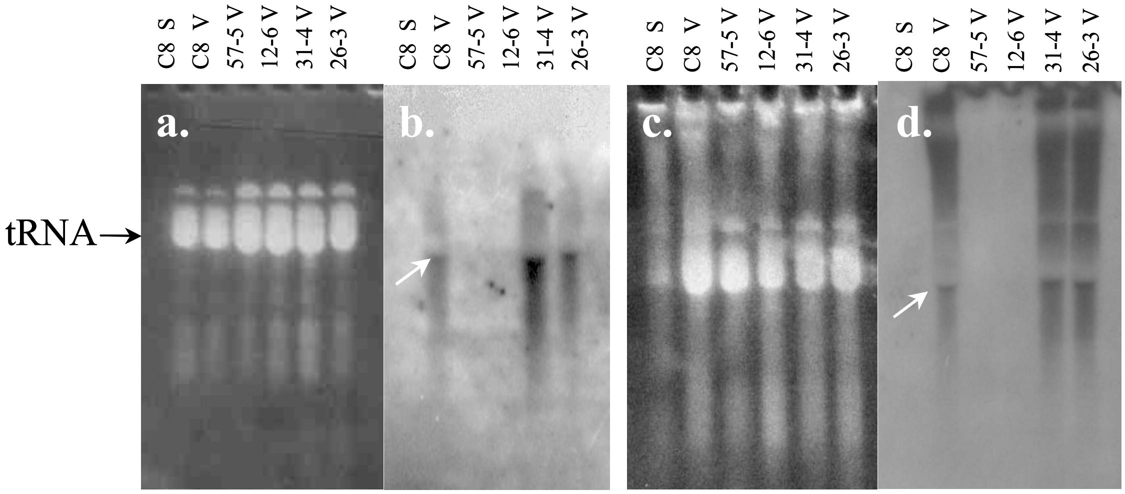

Figure 9. Extraction of small RNAs run on polyacrylamide gels. A & C are stained with ethidium bromide and B & D are the respective northern hybridizations with a probe specific to the N-gene on RNA3 of RHBV. For A & B, the small RNA were extracted using a method of Hamilton & Baulcombe; and for C & D the method of extraction was by Papaefthimiou. The lanes are Cica 8 healthy C8 S; Cica 8 infected with RHBV C8 V; and theother lanes are transgenic rice lines containing the RHBV N-gene.

OUTPUT 2. CHARACTERIZING RICE PESTS AND THE GENETICS OF RESISTANCE

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

- RHBV (Rice Hoja Blanca Virus) Nucleoprotein Gene Expression and its Association with RNA Mediated Cross Protection in Transgenic Rice

L. Fory, T. Agrono, C. Dorado, and Z. Lentini.

Introduction

Rice hoja blanca virus (RHBV) is a major virus disease of economic importance affecting rice in northern South America, Central America and the Caribbean. Previous reports described the generation and selection under field conditions of transgenic RHBV resistant Cica 8 plants carrying the nucleoprotein (RHBV-N) viral gene. The transgenic RHBV resistance encoded by the RHBV-N gene appears to be RNA mediated, where plants do not expressed the transgenic protein but low levels of RNA (Lentini et al., 2002). One potential mechanism underlying the transgenic resistance is through gene silencing. There is increased evidence that transgene expression in plants is characteristically unpredictable, and depends on many factors, including the position of integration, the number of transgene copies and the structural integrity of the integrated DNA. Transgene silencing can occur at the transcriptional or post-transcriptional level. DNA methylation is often associated with transgene silencing, although it is presently unclear whether methylation causes silencing or is a consequence (Ingelbrecht et al., 1994; Fu et al., 2000). Last year, we reported the evaluation in the field for two semesters of transgenic Cica 8 lines were forty five entries derived from line A3-49-60-12-3-3 were highly resistant showing scores 1 to 3. The transgenic resistance is stably inherited in crosses with other varieties (Annual Report 2001), and currently F_4 generation plants are being evaluated for yield potential and other agronomic traits to incorporate the best lines into breeding. This year we report the studies directed to understand the resistance mechanism underlying RHBV transgenic resistance via analyzing the RHBV-N gene expression in transgenic resistant plants.

Materials and Methods

Southern blot analysis. Two transgenic Cica 8 lines with known resistance (A3-49-60-12-3-57 resistant, and A3-49-60-12-3-1-31 susceptible) were selected. Genomic DNA was isolated from young leaf tissues (McCouch et al., 1988), and 20 µg DNA were digested with restriction endonucleases (Bam HI and KPN I), restriction fragments were resolved through 0.8% agarose gels, transferred onto Hybond-N^+ membranes (Amersham), and hybridized with Alpha-[32P]-dATP-labeled hybridization probe specific to the RHBV-N gene prepared using the multiprime DNA-labeling system (Amersham) (Sambrook et al., 1989).

DNA Methylation Analyses – PCR Technique. Total genomic DNA was extracted at 20 days after germination from individual plant using a Dnasy plant mini kit (Qiagen), and 200 ng of DNA was digested for 5 hr at 37°c with 30 units of restriction endonucleases methylation sensitive or insensitive. Two sets of primers were used. The first set amplified the complete RHBV-N coding region. The second set amplified a 2.0 kb fragment including the whole transgene, from the promoter to the terminal region (35S promoter-RHBV-N coding region-nos terminal) (Figure 1). All PCR products were separated by size on a 1.5% agarose gel stained with ethidium bromide. The fragments were transferred onto Hybond N^{+} membranes. The probe was synthesized by random-primer, and labeled by nick translation, in the presence of alfa^{32} dATP. Hybridization and high stringency washing were carried out according to standard procedures (Sambrook et al., 1989).

Results and Discussion

Southern Blot Analysis. The Southern analysis of genomic DNA showed multiple N gene fragments larger than the 1.4 kb expected size indicating that the transforming DNA did not integrate as a complete unit in the A3-49-60-12-3-3 line. The multiple banding patterns for the N gene suggest integrative fragmentation and rearrangements. The complex multiple banding pattern has been inherited through seven generations of selfing suggesting the integration at one locus. The detection of the corresponding 1.4 kb N sequence by PCR and RT-PCR, suggests the presence of at least one copy of the full length N gene sequence and the inability to detect the corresponding 1.4 kb N fragment by southern appears to indicate the lost of a restriction site(s) which could be due to methylation at one or both Bam HI/Kpn I flanking sites of the N gene sequence. Bam HI and Kpn I are inhibited by methylation of cytosine. Additionally, Kpn I is also inhibited when methylated at the adenine. Bam HI does not cut $GGAT^{m4}CC$; $GGAT^{m5}CC$; and $GGAT^{hm5}C^{hm5}C$. Kpn I does not cut $GGT^{m6}A^{m5}CC$; and $GGTAC^{m4}C$. If one assumes that the Kpn I and Bam HI sites are methylated, a tandem direct repeat is predicted at 5.4 and 5.9 kilobases, and tandem inverted repeat is predicted at 5.4 and 7.4 kilobases, respectively (Lentini et al., 2002).

Methylation Analyses – PCR Technique. Polymorphism was observed between the transgenic resistant and susceptible line sister lines when genomic DNA was digested with methylation sensitive or insensitive restriction enzymes (Hae III, Bg II, PstI and Dpn I) and then amplified by PCR (Figure 2A). These results suggest possible differential DNA methylation between these two lines. Progeny plants derived from each of these transgenic lines need to be evaluated to elucidate if these polymorphic restriction patterns are reproducible and associated with the resistance/susceptible trait. The original plasmid DNA was also analyzed following the same procedure. As expected, when the restriction enzymes cut inside the RHBV-N gene (Bam HI, Bg II, Pst I) and then the plasmid –DNA was subject to PCR using a set of primers flanking the RHBV-N coding region (sk and RHBV 10, Figure 2A), no RHBV-N fragment was amplified (Figure 2B). In contrast, when plasmid –DNA was digested with enzymes that did not cut within the gene cassette (ClaI) or cut outside the PCR amplified region (Eco RV and SphI), the complete RHBV-N was amplified using the same set of primers (Figure 2B). However, when restriction isoschizomer enzyme pairs (Mbo I and Sau3aI, Figure 2) were used, a differential amplification pattern was noted (Figure 2B). Mbo I cut the methylated séquence GATm5C but did not cut the Gm6AT site. Sau3A1 does not cut GATm5C but does cut Gm6AT. No plasmid-DNA digestion was obtained when Mbo I while Sau3AI did cut the DNA. These results suggest a possible methylation at the adenine. It is important to determine the level of methylation of the plasmid-DNA sequence used for the transformation in order to determine the methylation pattern that we hypothesis is occurring in the RHBV-resistant/ susceptible transgenic rice plants. Fu et al. (2000) used a similar strategy in rice and identified at least three distinct types of silencing effects associated with different methylation patterns, including a novel form of transcriptional silencing involving methylation of cytosine residues only at non-conventional sites in the coding region.

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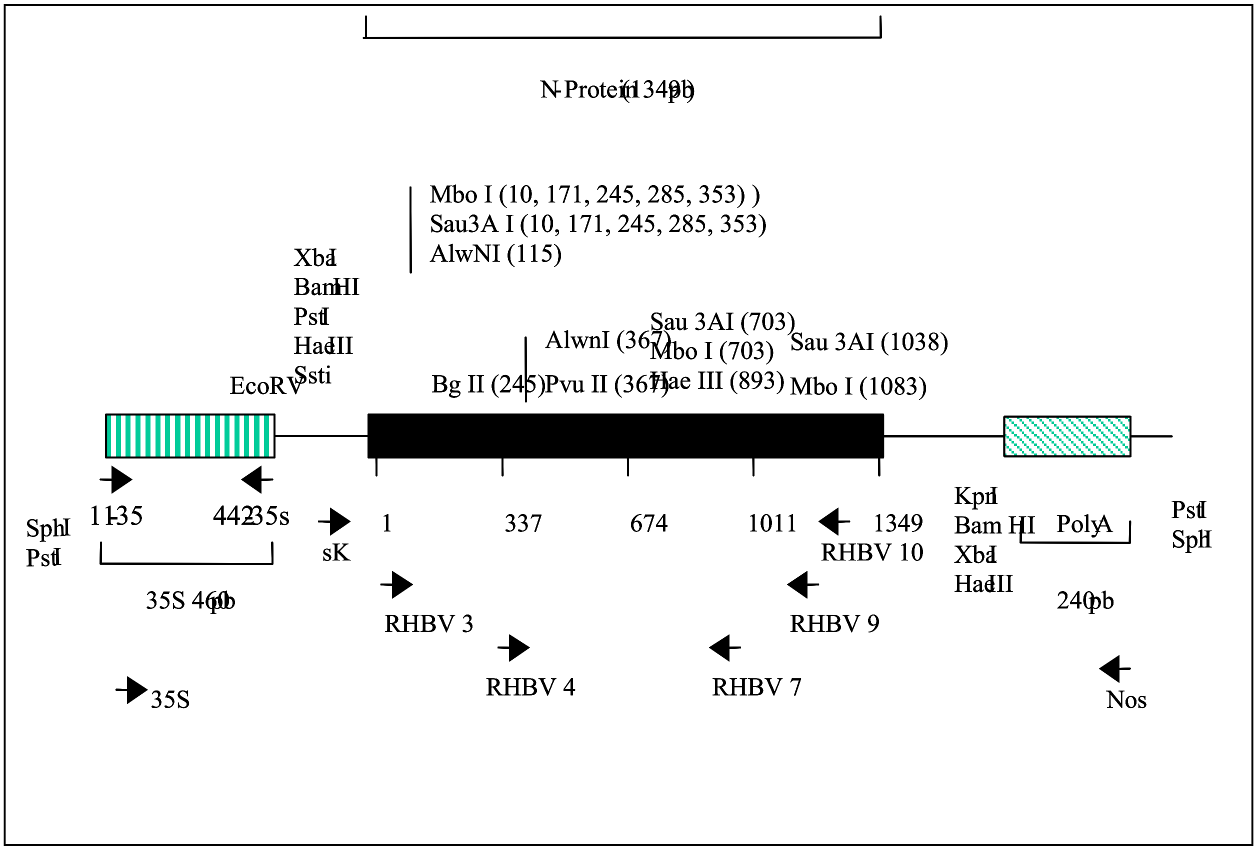

Figure 1. Map of Restriction Showing the RHBV N Gene. Using the Restriction Sites Kpn I and Sst I, the entire 1.4 kb inserted in the Sense Direction between the Cauliflower Mosaic CaMV 35 S Promoter and the NOS Polyadenilation. In detailed Show Direction, Sites Restriction and Primers used for Amplification of the Region Codificant, Promotor (11-35; 442-35S) and all Cassette (35S, Nos)

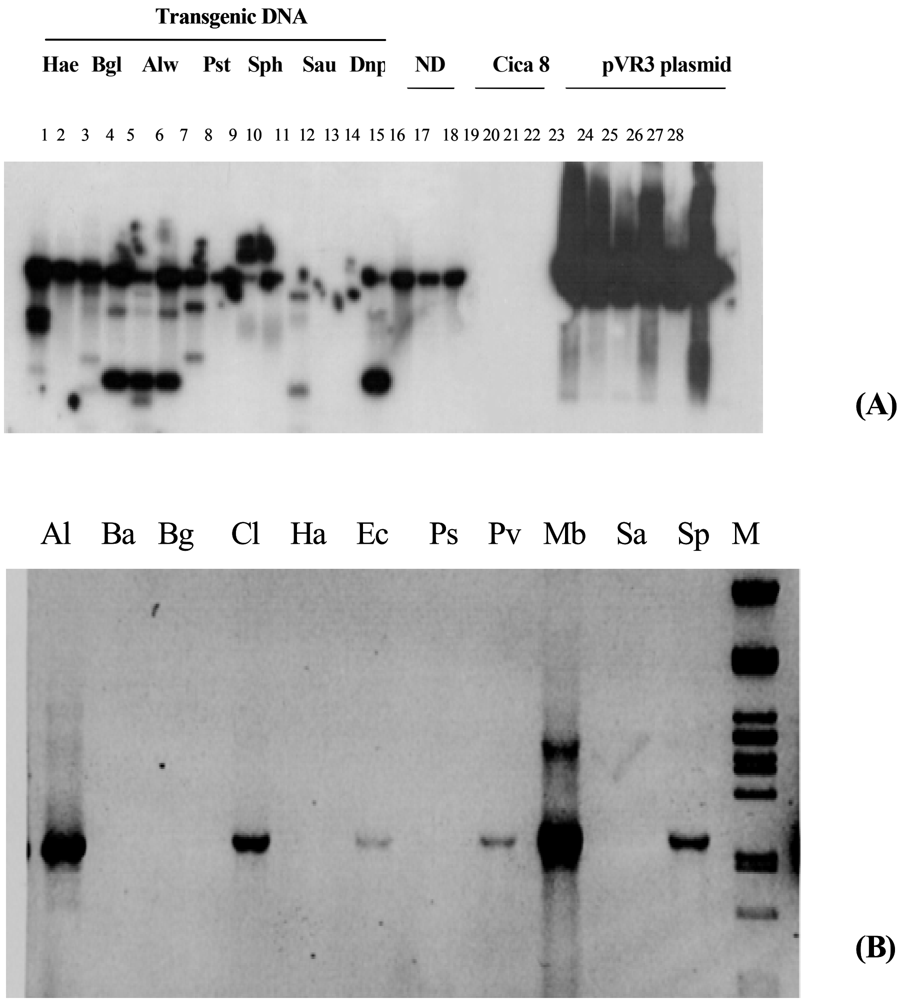

Figure 2. Southern Blot Analysis of DNA First Digested with Methylation Sensitive or Insensitive Restriction Enzymes Cutting Inside or Outside the RHBVN Gene Sequence, and the Amplified by PCR

(A) Genomic DNA of lines A3-49-12-3-3-57 (resistant) and A3-49-12-3-1-31(susceptible) was first digested with either of one these enzymes (Hae II, Bgl II, AleNI, PstI, SphI Sau3A1, or DpnI) or not digested and then amplified with a set of primers to amplified the complete RHBVN gene sequence including the promoter and terminal region of the gene. Lanes 1,3,5,7,9,11, and 13 correspond to A3-49-12-3-3-57 DNA. Lanes 2,4,6,8,10, 12, and 14 correspond to A3-49-60-12-3-1-31 DNA. Lanes 15-17 correspond to non digested transgenic DNA. Lanes 18-21 correspond to non-transgenic Cica 8 control. Lanes 22-28 correspond to the pVR3 plasmid.

(B) Plasmid *pVR3*DNA was first digested with restriction enzymes (Al) Alwn1; (Ba) Bam HI; (Bg) Bgl II; Cla I; (Ha) Hae III; (Ec) EcoRV; (Ps) Pst1; (Pv) Pvu II, (Mb) Mbo I, (Sa) Sau3A1(Sp) Sph I, and the then amplified by PCR. M. Ladder Marker λ

- **Characterization of Transgenic Rice Containing the RHBV Non-Structural 4 (NS_4) Gene from the RNA 4**

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Introduction

The genome of the Rice Hoja Blanca Virus (RHBV) consists of four species of Single Strand RNA (ssRNA) designated RNA 1, 2, 3 and 4. The RNA 4 encodes a major non-structural protein (NS_4), which accumulates in the tissues of plants infected with the RHBV. The differential plant-insect NS_4 expression, and the similarity of NS_4 sequence with well characterized helper proteins described for other insect-transmitted viruses, suggest that NS_4 might be involved in the RHBV transmission from the plant to the plant-hopper, or in the virus movement from cell to cell. The main goal for the expression of the RNA 4 in transgenic rice is to determine the function of the major NS_4 protein and study the potential for a novel and different method of producing viral resistant plants. Last year, we reported the transformation efficiency and recovery of transgenic indica varieties and identified 55 transgenic plants carrying the NS_4 sense and anti-sense orientations by Southern analysis (Fory et al., 2001, CIAT SB-2 Annual Report). The analysis for the gus-intron, hpt, and NS_4 transgenes, RT-PCR and Northern analyses for the NS_4 of the first generation of transgenic (T_1) plants derived from selfing of the original transformed lines (T_0 generation) and identified as transgenics by Southern blot, indicated that not all T_1 plants inherited the three transgenes. These molecular analyses suggest that in some of the lines the NS_4 gene may had been integrated in a separate locus, allowing the elimination of the antibiotic resistance and/or marker genes through sexual crossing in advanced generations. The initial evaluations of RHBV resistance in the transgenic plants showed that most T_1 plants from transgenic Palmar line 4 transformed with plasmid pIC004 showed intermediate levels of resistance (Fory et al., 2001, CIAT SB-2 Annual Report, 2001). In this report we present the results for the evaluation of RHBV resistance in the first generation of transgenic Cica 8, Palmar, Cimarron, Fedearroz 50, Nipponbare and the breeding line CT11275 plants transformed with five constructs (pIC002, pIC004, pIC007, pIC008 and pIC009), which differ in the NS_4 sense and anti-sense orientation, and the promoter sequence (35S CaMV promoter Vs maize ubiquitin promoter).

Materials and Methods

Rice Genetic Transformation. The genetic transformation was conducted following the procedure described in Fory et al., (CIAT SB2 Annual Report 2001), using the plasmid constructs described in Tabares et al. (CIAT SB2 Annual Report 2000).

Molecular Analyses of the Transgenic Rice Plants. Southern blot, PCR, RT-PCR, and Northern analysis were conducted as described in Fory et al., (CIAT SB2 Annual Report 2001).

RHBV resistance assays. Fourteen independent events of transgenic lines represented by 16-18 plants each per line was evaluated for RHBV resistance as described in Fory et al.

(CIAT SB2 Annual Report 2001). In all cases, only transgenic plants showing non-rearranged single integration NS_4 gene copy by Southern blot were selected for RHBV resistance evaluations. Cica 8 plants were inoculated at 15 days after planting, while Palmar plants were inoculated at 13 days after planting since the non-transgenic plants of this variety show intermediate RHBV resistance. Evaluations were conducted once a week starting 5 days after removal of viruliferous insect vectors, up to 54 days after inoculation.

Results and Discussion

Cica 8 transgenic plants generated using the constructs pIC002, pIC007 and pIC009 were susceptible to the RHBV. Between 69 to 100% of the plants showed symptoms on more than 50% of the leaf area. The reaction to the virus was similar to that observed for pIC002-12 line, used as the susceptible control. In contrast, Cica 8 line 9 containing the anti-sense NS_4 gene from plasmid pIC008 showed about 30% of the plants with less than 10% of leaf area with disease symptoms (Table 1). In some of these plants, the integration of the NS_4 gene has been demonstrated using Southern blot and PCR. Similarly, Palmar line 4 transformed with anti-sense NS_4 gene derived from plasmid pIC004, showed a significant 63% reduction in the number of plants diseased respect to the non-transgenic control. In this case, 53 % of the transgenic Palmar line 4 showed little or no disease symptoms (<10% leaf area affected (Table 1). These results corroborate last year results where the same line showed 54% reduction in disease (Fory et al., CIAT SB-2 Annual Report, 2001). While susceptible plants expressed low levels of RNA from either the sense or antisense-NS_4 gene as indicated by RT-PCR analysis, resistant plants showed low expression of the NS_4 gene as revealed by regular Northern analysis (Fory et al., CIAT SB-2 Annual Report, 2001).

Future Activities

Molecular analyses of T_2 generation derived from resistant T_1 plants are currently in progress to identify plants containing and expressing the NS_4 gene, and determine the gene inheritance.

Selected plants will be evaluated for RHBV resistance and resistant ones self cross to generate fixed lines.

Lines will be evaluated in the field.

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Table 1. RHBV Resistance Evaluations of T_1 Transgenic Plants derived from Independent Transgenic T_0 Events Carrying the NS_4 Sense or Anti-Sense Gene

Genotype	Line	NS_4	Number Plants	% Plant Gus +	Leaf Area Affected (% Plants)	
					≤ 10	>10-100
Cica 8	pIC002-12	Sense del.	17	4	0	100
Cica 8	pIC007-4	Sense	16	ND	0	100
Cica 8	pIC007-5	Sense	16	ND	0	100
Cica 8	pIC009-16	Sense	16	ND	12	88
Cica 8	pIC009-4	Sense	16	ND	6	94
Cica 8	pIC008-8	@sense	16	ND	6	94
Cica 8	pIC008-9	@sense	16	ND	31	69
Cica 8	NT	None	16	-	0	100
Palmar	pIC007-1	Sense	15	ND	40	60
Palmar	pIC004-1	@sense	16	65	19	81
Palmar	pIC004-10	@sense	16	37	44	56
Palmar	pIC004-4	@sense	17	82	53	47
Palmar	pIC004-7	@sense	18	0	28	72
Palmar	pIC008-5	@sense	16	ND	37	62
Palmar	pIC008-6	@sense	16	ND	44	56
Palmar	NT	None	16	-	25	75

Cica 8 plants were inoculated at 13 days after planting in the greenhouse and Palmar plants at 15 days ND= Not determined, the plasmids: pIC007, pIC008 and pIC009 do not contain the gus gene. NT non-transformed

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

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Introduction

The protection of crops against pathogens is of the utmost importance in modern agriculture. Major crop production losses are registered in rice each year due to various crop diseases. The fungi *Rhizoctonia solani* (causative agent for sheath blight), Helminthosporium, Rhynchosporium, and Sarocladium have been implicated in causing important rice yield losses in the Southern cone of South America and increasing spreads have been reported in Colombia, Mexico and Venezuela. All rice varieties are susceptible to these diseases and there are no known sources of stable genetic resistance. An alternative approach for the management of the rice sheath blight disease is to introduce into the rice genome genes that encode proteins with antifungal activity. Datta et al. (2000) had reported the introduction of chitinase gene in some indica rice cultivars, however transgenic plants were not stably resistant to sheath blight in advanced generations. The genus Phytolocca is the source of a number of proteins whose antiviral and antifungal properties have been analyzed. These antiviral proteins are designated pokeweed antiviral protein (PAP) and inhibit the infection of RNA and DNA viruses representing a different plant viruses, such tobacco mosaic virus in plants (Chen et al., 1991) and animal viruses, including poliovirus (Ussery et al., 1977), herpes simplex virus (Aron and Irvin, 1980) and human immunodeficiency virus (Zarling et al., 1990). There has been growing interest in using single-chain RIPs, such as PAP, as the cytotoxic component of immunotoxins targeted to cancer cells. The work by the Dr. Tumer group of Rutgers showed that transgenic tobacco plants expressing PAP are resistant to virus from different groups (Wang, 1998). Mutated versions of PAP gene have potent antifungal activity (Zoubenko et al., 1997). Homozygous progeny of transgenic tobacco plants expressing these PAP genes displayed resistance to the fungal pathogen *R. solani*. Transgenic PAP potato showed protection against *Phytophtora infestans*, and transgenic PAP turf grass are resistant to various fungal pathogens. We are interested in constitutively expressing PAPy123 gene in transgenic plants in order to obtain sheath blight resistance in rice. Last year, we reported on the generation of indica varieties carrying the PAPy123 gene (Tabares et al., CIAT SB2 AR 2001), a new version of PAPy123 gene with a deletion of 3 nucleotides. The southern blot analysis indicated the recovery of various transgenic lines showing the integration of at least one copy of the gene without rearrangements, and the Western analysis indicated that about 50% of the plants showed gene expression of PAPy123 protein. Here we report on advanced generations with stable inheritance of gene expression of PAPy123, which will be evaluated for sheath blight resistance.

Materials and Methods

Molecular Analyses of PAPy123 Transgenic Rice Plants. Southern blot, PCR, RT-PCR, Northern and Western analyses were conducted as described in Tabares et al., (CIAT SB2 AR 2001).

Evaluation of Agronomic Traits in the Greenhouse. Agronomic traits were evaluated on plants grown to maturity in individual pots. The number of days to maturity was determined by scoring the number of days from sowing to flowering. At harvest time, the number of tillers and panicles per plant were counted. The percentage of fertility was determined. Plant height was measured from the base of the plant to the tip of the youngest fully expanded leaf. Non-transgenic Palmar and Cica 8 varieties were used as controls.

Results and Discussion

Stability in inheritance and expression of PAPy123 gene in transgenic rice plants Southern blot, PCR, RT-PCR, Northern, and Western analyses indicate the stable integration, inheritance and expression of the PAPy123 gene from T_0 to T_1 generation of some PAPy123 transgenic plants. Of the transgenic events evaluated, from 40% to 100% of the T_1 plants inherited the PAPy123 gene (Table 2). Higher level of PAPy123 gene expression was detected in Palmar transgenic lines respect to the Cica 8 lines (Table 2).

Agronomic Performance of PAP 123 transgenic T_1 Generation Plants

No significant differences in flowering and plant height were noted between transgenic plants and the corresponding non-transformed controls (Table 3). Most transgenic plants showed a higher number of tillers than the non-transformed controls, especially those derived from Cica 8. Fertility was affected in some Palmar transformed lines; lines with high fertility level could be selected (Table 3). For example, PAPy123 transgenic Palmar line 31 and PAPy123 transgenic Cica 8 line 12 showed comparable or lower sterility percentages when compared with their respective controls, while showing relative increases in the number of tillers.

Future Activities

Selection of T_2 generation transgenic plants based of stable inheritance and gene expression, and good performance of agronomic traits. Sheath blight resistance evaluation with the collaboration of the rice pathology group (F. Correa) of the selected T_2 plants.

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Table 2. hpt, npt II and PAP 123 Genes Inheritance, and PAPy123 Gene Expression in T_0 and T_1 Transgenic Plants

			T_o Plants					T_1 Plants		
			PCR [1]		PAPy123 gene[2]			PAPy123 gene[3]		
Genotype	Plasmid	T_0 Plant	Hpt	Npt II	PCR	S+	W+	% PCR+	RT-PCR	N+
Cica 8	NT-446	1	+	+	+	+	-	90	Nd	Nd
		3	+	+	+	+	-	90	Nd	Nd
		4	+	+	+	+	++	80	+	+
		8	+	+	+	+	Nd	89	Nd	-
		11	+	+	+	+	-	60	Nd	Nd
		12	-	-	-	Nd	-	40	Nd	Nd
		13	+	+	+	+	+	50	-	-
		14	+	+	+	+	+	50	-	-
		16	+	+	+	+	-	50	Nd	Nd
		25	+	+	+	+	Nd	60	Nd	Nd
		29	+	+	+	+	Nd	60	+	Nd
Cica 8	None	NT	-	-	-	-	-	-	-	-
Palmar	NT-446	4	+	+	+	+	++	40	-	-
		6	+	+	+	+	+	100	+	+
		8	+	+	+	+	++	90	++	+
		16	+	+	+	+	-	90	Nd	Nd
		22	+	+	+	+	++	50	++	+
		23	+	+	+	+	Nd	80	Nd	+
		27	+	+	+	+	++	67	++	+
		29	+	+	+	+	Nd	80	++	+
		31	+	+	-	+	Nd	100	Nd	Nd
		37	+	+	+	+	Nd	90	Nd	Nd
		38	+	+	+	+	Nd	80	Nd	Nd
		39	+	-	+	+	Nd	100	Nd	Nd
Palmar	None	NT	-	-	-	-	-	-	-	-

[1] PCR analysis to detect the transgenes in T_0 plants: hpt, hygromycin; nptII kanamicin.

[2] Evaluation of PAPy123 gene in T_0 plant, S = + Positive to Southern blot analysis. W= +positive to Western blot of PAPy123 gene.

[3] The PAP y123 gene was evaluated in 10 plant per each To mediate PCR. Some these plant were analyses mediate assay transcription RT-PCR and Northern blot, N= +positive to Northern blot of PAPy123 gene.
Northern blot and Western blot were scored based on the level of expression. (+) low; (++) intermediate; (+++) high. Nd= Not determined. NT = Non transgenic

Table 3. Agronomic Traits of T_1 Transgenic Plants derived from Dfferent T_0 Plants Carrying PAPy123 Gene in the Glasshouse

Genotype	Plasmid	Plant [1]	DTH[2]	Plant Height (cm)	Number Tills	Number Panicle	% Sterility [3]
Cica 8	NT-446	1	104 bcde[4]	94 ab	24 ab	24 ab	11 ef
		3	103 bcde	94 abc	22 abcd	22 abc	11 ef
		4	105 b	87 abcde	21 abcd	20 bcd	10 ef
		8	105 bc	90 abcd	24 ab	23 abc	9 ef
		11	102 defg	92 abcd	21 abcd	20 bcd	15 ef
		12	102 cdefg	92 abcd	25 a	25 a	6 ef
		13	103 cdefg	90 abcd	24 ab	24 ab	7 ef
		14	103 bcde	86 cdefg	23 abc	23 abc	8 ef
		16	105 b	88 bcde	21 abcd	21 abc	8 ef
		25	110 a	80 hfg	22 abcd	22 abc	12 ef
		29	104 bcd	90 abcd	22 abcd	22 abc	15 ef
Cica 8	NT-446	Mean	104 bcd	90 e	23 a	22 a	10 ef
Cica 8	None	NT	103 bcd	96 e	17 d	17 cd	7 def
Palmar	NT-446	4	101 efg	80 hfg	22 abcd	22 abc	10 ef
		6	103 bcdef	66 I	17 cd	17 d	39 bc
		8	103 bcdef	79 hg	20 abcd	20 cd	66 a
		16	101 efg	79 h	25 a	23 abc	22 cde
		22	103 bcde	80 hfg	22 abcd	24 abc	31 bcd
		23	102 defg	90 abcd	21 abcd	20 bcd	13 ef
		27	100 fg	89 abcd	23 abc	23 abc	21 def
		29	102 defg	91 abcd	22 abcd	23 abc	49 b
		31	100 g	87 bcdef	23 abc	23 abc	7 ef
		37	100 fg	85 dfghe	23 ab	23 abc	12 ef
		38	102 defg	82 fghe	22 abcd	22 abc	35 bcd
		39	100 g	93 abc	18 bcd	17 d	5 f
Palmar	NT-446	Mean	101 bcde	83 bcde	22 abcd	21	26 cde
Palmar	None	NT	103 bcde	88 bcde	20 abcd	21 abcd	4 f

[1] Ten plants were evaluated per each T_0 line, [2] Days to heading, [3] Percentage of sterility

[4] Means followed by the same letter are not significantly different Ryan-Einot-Gabriel-Welsch multiple range test (p>0.005).

OUTPUT 2. CHARACTERIZING RICE PESTS AND THE GENETICS OF RESISTANCE

2E. Characterization of Crinkling Disease. A Complex of *P. graminis* and Rice Stripe Necrotic Virus

R. Sedano and L. Calvert

Introduction

This study was undertaken to better understand the interaction between rice stripe necrotic virus (RSNV) and the plant. This was done by looking at the titer of the virus at different times, and in different tissues using both highly susceptible, intermediate resistant and highly resistant rice lines or their relatives. These results also have implications for using ELISA as a routine diagnostic tool.

Materials and Methods

The plants used in this study were varieties of *Oryza sativa* including Bluebonnet 50, Colombia XXI and Coprosem 1 as well as the related species of *O. glaberrima, O. rufipogon,* and *O. Barthii*. The leaves and roots were analyzed for virus titer using DAS-ELISA with an antiserum specific to RSNV. The inoculation of the plants was described in the IP-4 AR 2001, and the plants are germinated and grown in soils contaminated with *P. graminis* containing RSNV. All experiments were carried out in greenhouse conditions.

Results

Comparing the Virus Titer of RSNV in Different Rice Lines and Related Species. At each date, leaf samples for ten plants of Bluebonnet 50, Colombia XXI and Coprosem 1, and *O. glaberrima* were collected and tested by ELISA for RSNV. During the first 22 days after planting, there were no symptoms of crinkling disease on any of the plants, so the sampling was a random selection. At 43 on 90 days, the plants that had symptoms typical of crinkling disease were selected for testing.

Table 1. The Titer of RSNV in Several Lines of Rice at different Ages of the Plants

Material	13 dap[1]	22 dap	43 dap	90 dap
Bluebonnet 50	0.2	0.05	0.47	.
Colombia XXI	0.2	0.78	0.52	1.87
Coprosem 1	0.2	0.15	0.48	0.85
O. glaberrima	0.02	0.05	0.05	.
Control RSNV(+) plant	0.75	0.75	0.37	0.75
Negative control plant	0.05	0.02	0.04	0.05

1 Days after planting (DAP).

In Colombia XXI and Coprosem 1, RSNV was detected before the leaves had symptoms (Table 1). After the plants developed symptoms, the ELISA test became a more reliable, but there was a range of virus titers (Table 2). It has demonstrates that the virus is not evenly distributed in the leaves with typical symptoms of crinkling disease. For diagnostic testing, that several samples should be taken in order to have confidence in negative results.

Oryza glaberrima was always negative for the virus. The vector *P. graminis* can infect the roots of *O. glaberrima*, so it appears that the virus does not replicate in the plant. There is a suggestion the titer of the virus may be associated with resistance to the disease. Bluebonnet 50 and Croprosem 1, which have previously been rated as resistant, had lower incidence of crinkling disease in greenhouse studies and a lower titer for RSNV as compared with Oryzica 3, with is one of the most susceptible rice lines.

Table 2. The Titer of RSNV in the Leaves and Roots of different Rice Lines

Material[1]	Leaves		Roots
	with symptoms	without symptoms	
Bluebonnet 50	0.26 [0.02-0.47] (2)	0.12 [0.01-0.35] (3)	0.2 [0.02-0.37] (2)
Colombia XXI	0.64 [0.02-1.81] (4)	0.16 [0.02-0.85] (5)	0.35 [0.21-0.62] (3)
Coprosem 1	0.19 [0.02-0.36] (2)	0.08 [0.02-0.15] (3)	0.15 [0.01-0.3] (3)
Oryzica 3	0.55 [0.01-1.23] (3)	0.29 [0.01-0.77] (5)	0.31 [0.01-1.31] (4)
FL00468-6P-1-1P-M			0.17 [0.17-0.17] (2)
O. barthii		0.01-0.04 (1)	
O. rufipogon			[0.09-0.10] (2)
O. glaberrima		0.03 [0.01-0.09] (5)	[0.01-0.02] (2)
Control RSNV (-)		[0.00-0.07] (4)	
Control sintomatico RSNV(+)	0.5 [0.33-0.77] (3)		

1. The test was done 30 days after planting.

Comparing Dried and Fresh Tissue using ELISA for RSNV

There are many advantages to using dried materials in diagnostic testing. This study compared fresh leaves and dried leaves with typical symptoms of crinkling disease. In both types of samples, the virus was easily detected (Table 3). There was no correlation between the type of sample and the ELISA values. This confirms that dried leaves can be used in routine diagnosis of RSNV.

Table 3. A Comparison of the Ability to Detect RSNV using ELISA in Dry and Fresh Tissue

Materials[1]	Treatment[2]	
	Fresh Leaves	Dried Leaves
Coprosem 1	0.42[3]	0.85
Fedearroz2000	0.91	1.73
Lemont	1.41	1.64
Victoria 1	0.79	1.10
Victoria 2	1.92	2.21
FL00478-29P-11-1P-M (cod 103)	0.89	2.13
FL00562-7P-11-1P-M (cod 140)	1.53	1.28
FL00440-47P-18-1P-M (cod 18)	2.07	1.58
FL00468-6P-1-1P-M (cod 84)	1.24	2.13
O. rufipogon	2.64	1.51
Oryzica 3 (+)	1.11	1.55
Oryzica 3 Asintomático	0.05	0.07

[1] Symptomic leave were used except where noted.
[2] The tissue weight was 0.02g per sample.
[3] O.D. ELISA at 60'.

Discussion

The ELISA test is an efficient diagnostic method for the detection of RSNV. Dried samples can be used and this facilitates long distant shipping of suspected samples. Symptomatic leaves should be collected and dried rapidly using silicon gel. Because there is variation of virus concentration within plants, it is recommend to collect samples from several locations on the affected plant and to collect sample of 0.1 to 0.4 g.

This methodology also shows promise as a means to study resistance to the virus. Additional testing needs to be done to determine if there is a true correlation between resistance and virus titer. If confirmed, this could be used to screen segregating populations.

Acknowledgements

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

3A. Participatory Development of Rice for Poor Communities in Marginal Areas

• RHICO a New Rice Type for Confronting Food Insecurity in the Hillsides

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Abstract

In the hillsides, rice is stable in the diet of the poor. Therefore it is important to develop upland rice with cold tolerance to help confront food insecurity in the mid-altitude mountains. A participatory scheme allowing the farmers to select for the characteristics that the identify as important including acid soil, drought and cold tolerance, and for resistance to rice blast disease. A participatory process of evaluation led to the nomination of the varieties Cirad 446 and Cirad 447. They are the first upland varieties in America with earliness, high tolerance to cold, drought and soil acidity, and with resistance to blast. So they are the first varieties of a new type of Rice for HIllsides with COld tolerance, named RHICO type.

Introduction

The CIAT-CIRAD collaborative project targets resource poor farmers and uses participatory methodologies to test new technologies. This year, the project worked with communities in marginal areas of the mid-altitude hillsides and high rainfall coastal areas. In these areas, the populations are mainly ethnic minorities, for example in Colombia, the mid-altitude site the population is principally native Indians, and the site on the Pacific coast the population is main blacks. Rice is an important crop for both these communities and the short-term goal is to increase the production to a level where these regions are self-sufficient thereby increasing their food security.

Upland rice for hot areas has been grown for several centuries in West Africa and Brazil for example. It is the same for lowland rice for hot areas such as India or Tolima, Colombia. Also lowland rice for areas in which due to the latitude or altitude cold tolerance is essential, for example Chile and Nepal respectively. But the first upland varieties for cold area have not more than a decade of history in Madagascar (Déchanet *et al.* 1996).

The mid-altitude hillsides such as the highlands of Colombia constitute a marginal area for the upland rice crop because of the cold night time temperatures. Often the populations are predominately ethnic minorities as in Colombian Andes (Guambianos, Totoroes, Coconucos, etc.). In these areas, rice is the major part of the diet, and they have expressed their desire to have adapted rice varieties as a part of their cropping systems. The following activities focused on the development of upland rice in the Colombian Andes had been allowed by the financial support of the Foundation Aventis-Institute of France, CIRAD, and CIAT.

Materials and Methods

The participatory evaluation started with the evaluation of 17 F_8 lines from Valès'crosses in Madagascar (Table 1). The trials form a complete dispersal blocks design. The plot size was 2 m^2.

Table 1. F_8 Lines Evaluated in Participatory Trials

#	F_8 lines	Progenitors Female		Male
1	PRA 522-2-63-1-1	F9 PRA 8-F317-6-9-6-M	/	Irat 265-57-2
2	PRA 522-2-66-2-1	F9 PRA 8-F317-6-9-6-M	/	Irat 265-57-2
3	PRA 522-7-24-5-1	F9 PRA 8-F317-6-9-6-M	/	Irat 265-57-2
4	PRA 546-38-71-3-1	Irat 114	/	Irat 380
5	PRA 553-44-64-1-1	Irat 265-57-2	/	Jumli Marshi
6	PRA 553-45-6-2-1	Irat 265-57-2	/	Jumli Marshi
7	PRA 553-45-8-4-1	Irat 265-57-2	/	Jumli Marshi
8	PRA 553-45-8-5-1	Irat 265-57-2	/	Jumli Marshi
9	PRA 553-45-8-6-1	Irat 265-57-2	/	Jumli Marshi
10	PRA 553-45-8-7-1	Irat 265-57-2	/	Jumli Marshi
11	PRA 553-45-8-8-1	Irat 265-57-2	/	Jumli Marshi
12	PRA 565-46-34-3-1	Khonorallo	/	Irat 265-57-2
13	PRA 565-46-42-1-1	Khonorallo	/	Irat 265-57-2
14	PRA 565-46-42-2-1	Khonorallo	/	Irat 265-57-2
15	PRA 565-46-42-3-1	Khonorallo	/	Irat 265-57-2
16	PRA 565-46-64-1-1	Khonorallo	/	Irat 265-57-2
17	PRA 565-47-19-1-1	Khonorallo	/	Irat 265-57-2

Then the best lines were evaluated a second time in a participatory trial with dispersal blocks. The plot size was 10 m^2. The best lines were milled with the manual huller (Vales and Roa, 1999) and evaluated for taste in participatory trials. The brown rice of these lines was compared to the milled white rice. The best, or the two best lines were evaluated in a third trial in plots of 25 m^2. These were also used as demonstration plots.

These lines were evaluated for their partial resistance to rice blast. This was done by using a rice blast pathotype, that overcomes the complete resistance genes of these lines. Then these lines were included in the field the trial using that used this strain to evaluate the partial resistance. Finally the lines that were resistant to blast and selected by the farmers were nominated as varieties and the multiplication has begun during the process of their official registration.

Results and Discussion

During the first semester of 2001, due to the drought the only lines that were harvested were PRA553-44-64-1-1 and PRA553-45-8-8-1 from Irat 265-57-2 / Jumli Marshi. Despite the high risk of drought, a few farmers evaluated these lines in plots of 10 m^2. The yield of the plots was approximately 3t/ha.

A participatory trial for evaluation of the taste of these lines was carried out during a workshop with farmers. The brown rice of PRA553-45-8-8-1 is red due to the color of its pericarp. After cooking, it turned to dark red. The color and the particular taste of this rice were preferred, so the brown (red) rice of PRA553-45-8-8-1 was the preferred rice. In second place was the brown rice of PRA553-44-64-1-1, and milled white rice was selected in third place.

During the first semester of 2002 the lines PRA553-45-8-8-1 and PRA553-44-64-1-1 were evaluated for their resistance partial to blast. The susceptible check was well attacked and the PRA lines were immune in spite of a compatible strain inoculation.

During the first semester of 2002, a drought occurred. Both lines were planting in plots of 25m^2 and yielded adequately and demonstrated their tolerance to drought in 25 m^2 parcels of some producers. So these lines were nominated Cirad 446 (PRA553-45-8-8-1) and Cirad 447 (PRA553-44-64-1-1) and the Crops and Seeds Aceituno Ltd. is to produce the certified seeds for the first semester of 2003. The official registration of these new varieties is underway.

In Colombia the cycle of these varieties from sowing to harvest is 3 month in Santa Rosa Experimental Station, Meta (333 m asl and 4°N), 4 month in the Palmira Experimental Station (1,054 m, 5°N) and 5 month in the Popayan sub-exprimental station (1,750 m asl, 2.5°N). Cirad 446 and Cirad 447 are the first upland varieties in America with earliness, high tolerance to cold, drought and soil acidity, and with a high level of partial resistance to blast. They are the first varieties of a new type of Rice for Hillsides with Cold tolerance, named RHICO type.

Perspectives

This program is supported by the Aventis-Institut Foundation of France, and it will receive the financial support of Colciencias Foundation for the next 3 years. The addition of rice, which is a dietary stable, into the cropping systems of these communities, is expected to have a strong impact in confronting food insecurity in the Colombian hillsides.

Reference

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- ## Confronting Food Insecurity in the Rain Forest of the Coasts

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Abstract

Rice production has been decreasing on the Guapi river strands and had disappeared on the Timbiquí river strands, in the rain forest of the Colombian pacific coast. The rice crop is an important stable and it needed for food security of this region. From two surviving traditional varieties, Brilla Lola and Chino Grande at least 3 other traditional cultivars are recovered. Another six traditional varieties were tentatively identified, and these results need to be confirmed. These recovered traditional varieties are very important as genetic resource. Commercial lowland varieties Orizica Caribe 8 and Fedearroz 2000 were identified as adapted to this area and were accepted by the farmers. Four tones of seeds of these varieties were provided to augment rice production. The first participatory trials showed that the upland variety Irat 216 was adapted for upland and lowland conditions and was accepted by the farmers. This variety is known to have a salt tolerance and may be a widely adapted to a range of local conditions.

Introduction

Few years ago, due to development projects of oil palm tree and coconut tree production for the communities of the pacific coast of the Colombian Cauca Department, their traditional production of rice declined drastically. These development projects were unsuccessful because of a drastic decrease in the coconut price, and disease of the oil palm trees, so never harvested. As a consequence, rice of the Timbiqui river strands disappeared, and only two traditional varieties are maintained in few fields on the Guapi river strands. The main consequence is a growing demand for the important food crops. The town of Guapi imported 720 tones of white rice *per annum*. The resource poor rural community had even less access to the basic commodity rice. For food security, it is urgent to reactivate the rice crop on the Pacific Cost of Colombia.

The National Programs for Transfer of Technology (Spanish acronym PRONATTA) is a major Colombian organization that is working for the development of the pacific coast region of Colombia. It has provided three years financial support for the reactivation of the rice crop in this region.

- **Rescue of the Traditional Rice Varieties Lost: Recovery of Genetic Resources and Traditional Knowledge**

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Introduction

The objective of this work was the rescue of the traditional rice varieties. These varieties were the results of decades of traditional selection and are well adapted for the costal conditions of high rainfall and low light intensity as well as being the quality of rice preferred by these community. These traditional rice varieties are important genetic resources.

Material and Methods

On the Guapi river, only the traditional varieties Chino Grande and Brilla Lola are still grown. Due to an lack of organized seed production, these varieties were not uniform an appeared to be populations. It was a hypothesis that these rice populations were actually mixtures and they contained other traditional varieties.

Seeds from individual plants with different phenotypes were harvested in fields of Chino Grande and Brilla Lola. These extracted lines are fixed by pedigree selection on panicle lines. Participatory selection was made to determine which lines were recognized as traditional varieties by the local farmers.

Results

Forty one lines were extracted: 25 from Brilla Lola, and 16 from Chino Grande. The first results of the participatory identification trials are in the Table 2. From the traditional variety Brilla Lola several traditional varieties were identified including Callila, Chino Grande (barbon), ICA Alto, Fian, ICA 4, and Guacari. From the traditional variety Chino Grande several traditional varieties were identified including Chino Pequeño, Panameño, Negrito, Chino Chiquito, Bogotano and Arroz blanco. These results are still considered preliminary and additional test is being done to confirm that these are distinct varieties.

Discussion

Many different phenotypes were found within the traditional varieties of Brillo Lola and Chino Grande. We are waiting for the complete participatory trial information but some of the fixed lines probably will not be clearly identified by the farmers. This can occur because of the loss of traditional knowledge and some lines come from spontaneous crosses. The recovered traditional varieties are an importance as genetic resource, and they are being incorporated into a collection of progenitor lines to be used to breed new varieties for humid lowland conditions. These traditional varieties will to be included in the next set of agronomic trials as well as incorporated into the seed-banks for the costal area that will be maintained by farmers

Table 2. Identification of Lost Traditional Varieties

#	Identified as	
	Timbiqui(*)	**Guapi**(**)
	Lines from Brilla Lola fields	
1	Calilla	Ica Alto
3		
6		Fian
11		Ica Alto
17		Ica 4
22		Guacari
25	Chino Grande (barbon)	Guacari
	Lines from Chino Grande fields	
26	Chino Pequeño	Negrito
28	Panameño	Panameño
30	Chino Grande	
31		Chino Chiquito
33		Bogotano
34		Arroz blanco
39		Bogotano

(*) Participatory identification during a field day at maturity stage

(**) Participatory identification on seeds (the participatory identification during a field day at maturity is coming up).

- ### Evaluation of Commercial Varieties in the Pacific Coastal Region of Colombia

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Objectives

The rice producers of the lowland areas along the rivers of the pacific coastal region of Colombia know only their traditional varieties. These varieties are very well adapted for the areas in which the crops may be flooded, the temperatures are high but the illumination is low. But these varieties do not grow well in favorable upland conditions that are also prevalent in the region. The objective of this trial was for the farmers to compare their traditional varieties with other varieties of rice. This new knowledge will allow these farmers to have more choices.

Materials and Methods

During the first and second semester of 2001 (cycle 1 and 2 in Table 3) the varieties were evaluated in lowland conditions. These fields were frequently inundated because these rivers rise and fall with the sea tides. During a third cycle, first semester of 2002, these varieties were evaluated in lowland and in favorable upland conditions (Table 4).

Each participatory trial, the plot size was 10 m^2 by material and the planting density 46 kg/ha. The trials were planted in ten communities.

Table 3. Varieties Evaluated in Participatory Trials

Cycle	Name	Comment
1,2,3	Chino Grande MV 966(1)	Traditional lowland variety of this area, as check
1,2,3	Brilla Lola MV 957(1)	Traditional lowland variety of this area, as check
1,2,3	Ica 4	"Like traditional" lowland variety of this area, as check
1,2,3	Orizica Caribe 8	Known variety in this area, so reintroduced by the project
1,2,3	Orizica Yacu 9	Lowland variety
1,2,3	Orizica Turipana 7	Lowland variety
1,2,3	Fedearroz 50	Modern lowland variety
1,2,3	Fedearroz 2000	Modern lowland variety
3	Fedearroz Victoria I	Modern lowland variety
1,2,3	Fedearroz Victoria II	Modern lowland variety
1,2,3	Orizica Llano 4	Variety for favorable upland conditions
1,2,3	Orizica Llano 5	Variety for favorable upland conditions
1,2,3	Orizica Sabana	Upland variety
1,2,3	Orizica Sabana 10	Upland variety
1,2,3	Irat 13	Mutant of traditional upland variety of West Africa
1,2,3	Irat 216	Upland rice variety
3	Cirad 405	Upland variety
1,2,3	Cirad 409	Very early upland variety
3	Slip 72-M	Upland variety with long grain
3	PRA 546 (2)-38-71-3-1	Very early upland variety for cold hillsides, RHICO type
3	PRA 553 (3)-45-8-5-1	Very early upland variety for cold hillsides, RHICO type
3	PRA 565 (4)-46-42-2-1	Very early upland variety for cold hillsides, RHICO type

(1) Working collection number, so this variety was fixed by pedigree selection

(2) Irat 114 / Irat 380 ; (3) Irat 265-57-2 / Jumli Marshi ; (4) Khonorallo / Irat 265-57-2

Results and Discussion

During the first semester 2001, most of the trials were destroyed by very high water levels during of May, mainly along the Timbiquí river. The trials were planted again during the second semester 2001. Due to the traditional management, the fields are too heterogeneous for allowing a statistical interpretation of the results. Still relevant information about the general adaptation of the varieties and farmer's preference were determined. For example the Table 4 give a good illustration of the kind of information obtained from a participatory trial. All the traditional varieties are late, and the farmers had a preference for some of the earlier cultivars. The other preferences of the producers are: medium high plant for an easy harvest, good tillering, strong stems, numerous and long panicles.

This project introduced one ton of Oryzica Caribe 8 seeds to reinitiate the rice production on the Timbiquí river strands. This was done before the participatory trials, because the producers had in memory that this variety and it is adapted to this area. It was the only one commercial variety in the memory and the results confirm the producer knowledge. After the flooding in May 2001, three tons of Fedearroz 2000 seeds were sent to the area as it appears to be the best lowland variety accepted by the producer and with enough available seeds.

Table 4. Participatory Evaluation of Rice Varieties, San Miguel July 21, 2001

Varieties		Comments	
#	Name	UMATA technician	Producers
1	Ica 4	Well adaptation for wet lowlands, but poor growth in upland condition with dry and compact soil	Not accepted because the plants are too short, and their growth and development is slow
2	Orizica Caribe 8	Its development in dry land is very different. It is well adapted for lowland	Accepted, it is a known variety recognized for its yield and adaptability in the area
3	Fedearroz 50	Although it is a new variety in the area, it presents good tillering	Accepted by the producers for a possible sowing for its architecture, tillering (the producers are waiting to know its production)
4	Fedearroz Victoria II	Good development and a height acceptable	Accepted by the producers for a possible sowing for its high number of panicles and is moderate height
5	Orizica Llanos 5	Good architecture and excellent tillering	Possible use because it is very well adaptated for the lowland conditions (the producers are waiting to know its production)
6	Irat 216	It is very well for vegetative development and panicle formation	Accepted to have long panicles
7	Chino Grande	Very good tillering	Accepted to be a native variety for their architecture and vegetative development
8	Brilla Lola	Needs lot of humidity. For this reason some time the plants are too short	Accepted to be native, the producer continue the grow of this variety
9	Orizica Sabana 6	Good number of panicles. This upland variety is good for humid lowland	Accepted
10	Orizica Sabana 10	Good tillering	Accepted. Preferred for their strong stems
11	Orizica Yacu 9	Short plant (blooming stage	Not accepted because the plants are too short for an easy manual harvest
12	Irat 13	Good vegetative development and panicle number	Accepted because it likes for warm period and upland conditions
13	Cirad 409	Earlier variety	Accepted for its earliness
14	Orizica Llanos 4	It is a dwarf variety with a bad architecture	Not accepted to be a dwarf variety
15	Orizica Turipana 7	Blooming stage	Not accepted because too late

The surprise was the good adaptation and acceptability of some upland varieties in rain forest. In the third cycle of the participatory trials, some were carried out in favorable upland conditions. IRAT 216 was frequently selected by the farmers. This is an upland variety is performs well under these lowland conditions. Also, it has some tolerance to salt toxicity (Clement, 2001, person. com.), so it may be possible to grow this variety on the strands in the lower part of the rivers.

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

3B. Rice and Sorghum Participatory Plant Breeding in Central America

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Introduction

This new collaborative research project between CIAT and CIRAD was first discussed and agreed upon during the fifth CIO/CIAT meeting held in Cali in June 2001. It was further strengthened by discussions between CIAT and CIRAD-CA (first proposal of a concept-note of the project) and by the visit of Dr. Gilles Trouche to Nicaragua, Honduras and CIAT's Headquarters in November 2001. The project began in April 2002 with the signature of a Memorandum Of Understanding between CIAT and CIRAD-Ca and further outposting of Dr. Gilles Trouche in Managua, Nicaragua. The project proposes to develop participatory variety selection (PVS) and participatory plant breeding (PPB) approaches for two « model plants » -- i.e., rice and sorghum – for small and medium-scale farmers undergoing progressive crop intensification and increasing access to markets.

Upland rice, otherwise known as aerobic rice, is a developing staple crop in several Central American and Caribbean countries, mainly in plains with high rainfall (1300-1500 mm per year) but also on hillsides as a component of diversified cropping systems. The total area of rice, upland and irrigated, in Central America and the Caribbean totalizes 630,000 ha with a global paddy production of approx. 2400,000 tons (FAO, 2002). In Nicaragua, aerobic rice covers 55,000 of the 83,000 ha which makes up the average total rice area (MAGFOR, 2002).

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

The project aims to further develop methodologies of PPB and to improve breeding material. The specific objectives of the project are two-fold:

- The PPB work will be conducted in cooperation with existing farmer organizations and other relevant local actors. It is through these organizations that the activities of the project will be organized.

- The PPB will be based on the genetic enhancement of locally adapted populations with a broad and/or narrow genetic base, mainly through recurrent selection but also through more conventional breeding methods.

For rice, segregating material and fixed lines derived from populations and crosses developed by the CIAT Rice Project will be used.

For sorghum, it is proposed that introduced segregating and fixed material from CIRAD West-Africa Breeding Programs will be used. This material is genetically and morphologically diversified. Other available local improved breeding materials (from INTSORMIL and regional NARS) will also be used. All this material would first be evaluated together with farmers for adaptation to local cropping systems. Special emphasis will be placed on environmental adaptation (response to photoperiod, drought and pest tolerance) and on grain and forage quality. Existing composite populations developed from African germplasm may be further enhanced using better local materials to correspond to local constraints and production objectives.

Participatory and decentralized plant breeding is a breeding strategy which addresses needs and preferences of small-scale farmers in marginal areas where conventional plant breeding had little success. PPB proposes to involve farmers and other product users of a specific crop in all stages of a breeding program. Decentralization would ensure that the specific conditions of the target environment (climate, soils, agronomic practices …) are respected, in order to better control genotype by environment interactions, which are often very high in traditional cropping systems in marginal areas. PPB goals may be 1) gains in productivity, and a higher product value through quality increment 2) better effectiveness of breeding work because of effective targeting of user needs and production conditions 3) biodiversity enhancement and dynamic conservation of diversity 4) capacity building and knowledge generation for farmer communities and formal research (Sperling et al., 2001). Stakeholders participation in a PPB program can be characterized depending on the stages of participation during the process development of the new varieties, their degree of participation and the role of the different actors (Sperling et al., 2001). In PPB, we used to distinguish Participatory Varietal Selection (PVS) in which farmers select for fixed lines or varieties and Participatory Plant Breeding (PPB), in which farmers participate in the selection of segregating material (Witcombe et al., 1996).

General Problems

Rice blast (*Pyricularia grisea* Sacc.) is one the major constraints to upland rice production in Central America. Other main constraints for rice production enhancement identified in Nicaragua and Honduras are: a lack of improved varieties which are adapted to the diverse farming systems, insufficient weed control, drought, unsatisfactory grain quality for industry requirements and competitive pressure from imported rice.

Factors that limit sorghum yields and farmers' gains include drought, low soil fertility, pests (midge, head bugs, fall armyworm) and diseases, as well as low straw quality and little improved varieties offer.

Materials and Methods

Identification of Sites and Partners

This first step, necessary for adjusting and implementing this project, started during the first trip in November 2001, to Nicaragua and Honduras. During these first five months of implementation of the project activities, we focused our activities on these two countries. Emphasize was placed on Nicaragua, the actual project location, because of the country's greater diversity of agroclimatic and institutional environments for both rice and sorghum.

For the identification of sites, existing literature from different sources was reviewed: national agricultural statistics, research publications from NARS, CIAT and other institutions, studies from NGO and projects etc. Additionally, interviews and meetings were conducted with key informants such as national researchers, CIAT staff, extension services and NGO leaders, in order to verify and complete the former information. Field visits in the regions during the cropping season have also been frequently organized.

For the identification of partners, not only CIAT's long experience and institutional relationships in the region were used but also CIRAD's experience and relationships in Nicaragua developed during the 90's with project such as the "Programa Regional de Reforzamiento a la Investigación Agronómica sobre los Granos en Centroamérica" (PRIAG). Regarding possible NGOs as partner, we mainly looked for local organizations having a great experience in technical or/and financial support to small and medium-scale farmers in one or both crops, in training for farmer experimentation and in farmers' organization capacity building and NGOs which work with networks of farmers in the project area.

Diagnostic on Crop Systems and Farmers' Variety Needs

This activity comprises diagnostic to characterize the rice and sorghum cropping systems as well as farmers' variety needs. For this purpose, several meetings and workshops were organized with farmer groups in the study area.

Regarding rice, knowledge on farmers' needs and results of participatory evaluation of new rice varieties which have been underway since 1998-99 are available for the hillsides areas of Yorito in Honduras and San Dionisio in Nicaragua, where the CIAT hillsides project PE-3 has been working for many years in participatory evaluation of new germplasm in relationship with the CIALs farmer committees. In these regions, rice is considered as an alternative staple crop to the two predominant crops, bean and maize. Outside these areas, three meetings were organized jointly with INTA and/or the NGO NITLAPAN with rice growers in upland rice areas of Rivas (Ochomogo and San Juan Viejo villages), Jalapa and Masaya. Three other meetings are programmed in collaboration with NGOs for September and October in the rice growing areas of Chinandega, Quilali and Waslala.

For sorghum, very few recent data about constraints of cropping systems and farmers' variety needs are available in the existing literature. Therefore, it was considered necessary to conduce a more complete diagnostic study at the beginning of this project. The diagnostics will be carried out by two students from CNEARC Tropical Agronomy School (France) and UCA University (Nicaragua) in one representative sorghum production area with small and medium-scale farmers in Nicaragua. The first thesis work is been conducing by Felipe Martinez to obtain a master of science in tropical agronomy. The thesis work comprises a participatory diagnostic of sorghum cropping systems and variety diversity used and proposals for developing a participatory breeding program for the Madriz department area, in Esteli region (North of Nicaragua). This study is being carried out in collaboration with the NGO INSFOP/UNICAM in four farmer communities which represent different sorghum production conditions in these semi-arid hillsides in relation to climate, soils, topography, ethnic skills, social organization and institutional environment. The second thesis work seeks to improve the understanding of the cultural practices, constraints (abiotic and biotic) and potentiality for different sorghum cropping systems (including photoperiod-sensitive and insensitive sorghum type) in three farmer communities of the same region. For the first study, workshops were organized in each community in order to get general information about the community (natural resource map, social classification). Information was also gathered on the history of the sorghum crop, existing cropping systems, present and old varieties used and farmers' needs to improve sorghum production and use. Individual semi-structured interviews with 10 to 12 farmers and focus interviews with key informants were held in each community. Following the workshops, local sorghum varieties were collected from each community. The main part of these varieties were planted in one of the community for a participatory characterization and classification of these with farmers.

For the second thesis, about 40 sorghum plots will be studied during the two growing seasons of sorghum, *primera* and *postrera* in the three selected communities.

In other regions of Nicaragua, three meetings with sorghum producers have been organized in collaboration with local NGO or with the CIALs in Somotillo (one of the driest region of Nicaragua), in Ciudad Dario and in San Dionisio. During each meeting, a rapid participatory diagnostic on sorghum cropping systems, utilization of grain and stover, existing varieties and farmers' needs was conducted, which permitted to determine objectives, conditions and choice of the varieties for the PVS trials to be proposed during this first year. Two meetings have also been organized with farmer groups in Honduras, in the El Paraiso department in collaboration with the NGO Movimondo and in Yorito in collaboration with IPCA and the CIALs.

Plant Materials

Evaluation of rice and sorghum germplasm introduced from different sources:

Rice

Observation Nurseries

- 323 CIAT progenies from CIAT/Peru program and from interspecific crosses *Oryza sativa* x *O. glaberrima*, *O. sativa* x *O. barthii* and *O. sativa* x *O. rufipogon* for lowland irrigated conditions or favorable aerobic conditions (from Cesar Martinez program).
- 24 F_4 CIAT-CIRAD progenies derived from PCT-4 population for aerobic upland conditions (from Marc Chatel program).
- 5 CIAT-CIRAD varieties for upland conditions: CIRAD 409, 446, 447, ORYZICA Sabana 6, ORYZICA Sabana 10
- 6 CIAT varieties for irrigated conditions : Fedearroz 50, Fedearroz 2000, Bg90-2, Oryzica 1, Selecta 320 and Oryzica 3

Participatory Breeding

- PCT-18 : narrow genetic base population adapted for upland conditions for rice blast resistance and grain quality (from Michel Vales program).
- PCT-17 : narrow genetic base population adapted for high altitude hillsides upland conditions for rice blast resistance, cold tolerance and grain quality (from Michel Vales program).

Participatory Varietal Selection

- 14 advanced lines and varieties from INTA Rice Program for upland and lowland conditions.
- 9 CIRAD varieties from collaborative program in Ivory Coast, Brazil and Madagascar for less favorable upland conditions.
- 15 CIAT-CIRAD advanced lines for high altitude hillsides.

Sorghum

Observation Nurseries

- 21 photoperiod sensitive landraces and improved lines from CIRAD germplasm collection (late-flowering core from different origin Cameroon, Chad, Mali, Burkina, Sudan).
- 30 fixed lines, landraces and varieties from CIRAD-INERA program in Burkina Faso and other African origins with drought tolerance, early-flowering, foliar diseases and midge and bugs resistance or double purpose use.
- 13 forage sorghum and pearl millet advanced lines and varieties from ICRISAT-CIAT program in Colombia.

Participatory Varietal Selection

- 22 improved varieties from different CIRAD collaborative programs in West Africa (14 from joint CIRAD-INERA program in Burkina Faso) with drought tolerance, foliar diseases and sorghum midge and bugs resistance or double purpose use.
- 5 improved varieties from INTA.

Methods

Observation nurseries were planted on INTA experimental stations in Managua (CNIA), Posoltega (upland rice) and Sebaco (irrigated rice) and at the CIAT referential site SOL for agriculture hillsides of San Dionisio in Nicaragua. PVS trials and PPB work were conduced at the referential sites SOL of San Dionisio and Yorito (Honduras) and in farmer fields in Madriz, Rivas and Chinandega departments in Nicaragua.

For rice, one observation nursery with 30 lines and varieties was planted on the Posoltega INTA experimental station. Another nursery will be planted during the 2002-2003 dry season with 342 F_6 diversified lines for lowland conditions on INTA experimental station in Sebaco valley. Three PVS trials were planted at the CIAT referential sites of San Dionisio (low hillsides) and Yorito (medium and high hillsides) with respectively 12, 7 and 12 entries. Two rice populations were sown at San Dionisio (PCT-18) and Yorito (PCT-17) to start a PPB work with local CIALs. Two on-farm PVS trials were planted in the Rivas region with both 18 entries. The designs used for PVS trials were randomized complete blocks with two o three replications.

For sorghum, four nurseries were planted for observation and seed multiplication in Nicaragua, respectively on the Managua CNIA station, at the San Dionisio referential site SOL (2 nurseries) and at an INTA site of experimentation in Madriz during the *primera* and *postrera* seasons, with respectively 51, 11, 28 and 20 lines and varieties, including local checks. Seven PVS trials were planted during the second growing season (*postrera*), each one trial at the referential sites SOL of San Dionisio and Yorito, while the remaining four were conducted on-farm in the five different communities of Nicaragua and Honduras in which workshops and rapid participatory diagnostic of variety needs with local farmer groups have been previously carried out. The designs used for PVS trials were randomized complete blocks with two replications.

Preliminary Results (April to September 2002)

Identification of Partners and Sites

For both Nicaragua and Honduras, the CIAL farmer committees in San Dionisio and Yorito will be partners for both crops. Rice and sorghum are an alternative food crop to maize and bean in these mountainous areas. In both countries, CIALs have been involved since 1999 in participatory evaluation and selection of new upland rice varieties. Moreover, in the San Dionisio area, the NGO Prodessa had previously carried out participatory on-farm trials for rice varieties since the early nineties, which has led to the adoption by farmers of two improved IRAT varieties. Possible partners in San Dionisio and Yorito include local NGOs and farmer organizations already involved in the SOL and CIALs processes such as Campo Verde y ADDAC in San Dionisio and IPCA and Sertedeso in Yorito.

INTA is considered a strong, responsible partner in Nicaragua for the research area because it manages research programs both for rice and sorghum and recently concluded a general agreement of scientific cooperation with CIAT. A specific agreement for the project has been written and will most likely be completed between INTA and CIAT before October.

Other project partners in Nicaragua will be the NGOs NITLAPAN and UNICAM/INSFOP. NITLAPAN is the first provider of micro-credit for small and medium-scale farmers in Nicaragua. Besides providing micro-finance, NITLAPAN also supplies training and technical assistance to a wide network of farmers. In collaboration with this NGO, four meetings with farmers had been carried out while three rice and sorghum PVS trials were co-managed at two representative sites of sorghum and rice production areas. A specific agreement between CIAT and NITLAPAN is currently under discussion. For the dry hillside areas of North of Nicaragua, we have identified the local NGO UNICAM as a strong partner for the sorghum activities. Since 1996, UNICAM has been offering training and technical support to experimental farmers groups, which are known as CPEC. UNICAM works in 11 villages and with 70 communities representing 1200 families. It is a very active promoter of farmers exchanging knowledge and experiences, organizing every year in Nicaragua a Central American meeting for innovative farmers. Developing the PPB activities with the CPEC network could probably lead to a rapid and major impact. In 2002, UNICAM is involved in the diagnostic work on sorghum cropping systems and variety diversity, as well as the sorghum PVS trials carried out in the Madriz department.

For Honduras, we have identified the NGO Movimondo and the Alauca area as probable partner and site for sorghum activities. The traditional rice area of Comayagua department has been identified as an appropriate region to conduce PPB rice activities; its proximity from Yorito area will permit to take profit and to authorize a better impact of the results here obtained because, contrary to Yorito area, the Comayagua area is an old and present rice area where exist a great farmer knowledge of the crop and functional plants for transforming and commercializing rice.

Diagnostic on Crop Systems and Farmers' Variety Needs

Rice

In the traditional upland rice area of Rivas in the Pacific plains of Nicaragua, rice covers about 2,000 hectares. Production is mainly carried out by small and medium-scale farmers. Meetings with rice producers groups in Ochomogo and San Juan Viejo have helped to identify soils fertility, rice blast, bugs, climatic conditions (rainfall irregularity, drought and drying winds in November), access to credit and selling prices as major constraints to rice production in the area. Implementation of modern improved varieties of rice from INTA or ANAR (Asociacion Nacional of Arroceros) is generally low and depends on the level of intensification and the institutional environment. In Ochomogo, with the influence of the local farmer cooperative which exploits 70 hectares for rice production with irrigation, upland rice producers of this area know and partially use modern varieties. In San Juan Viejo, farmers use two modern varieties, limited on the flattest areas with better clay soils, but still use four local or ancient improved varieties, more rustic, to plant on the hillside less fertile soils. Farmers ask for new varieties that will provide plants with intermediate height (80-100 cm), early-flowering (90-100 days to harvest), resistant to blast rice and bugs, less fertilizer consuming (in comparison with INTA modern varieties), good grain and straw yields and adequate grain quality for market. Both villages are very interested in testing new rice varieties within their own environments.

In the wet, fertile Jalapa valley in North of Nicaragua, upland rice is an important and traditional crop, the third most cultivated after maize and bean. In the 1980s, rice cultivation in the valley reached 3,300 hectares (1982) and then decreased to 1750 ha in the 1990s and 1000 ha in 2002 because of lack of credit, problems of land property and low prices on the national market. Because of a government program introduced in 2001 to increase prices paid to the farmers, in order to boost the national production of rice (PAPA program), prices are now more incentive for rice producers. In this area, rice production is carried out by medium and large-scale farmers and firms. Blast rice and red rice are the main production constraints, while Cerscosporium and Rhyncosporium diseases appear to be secondary constraints. Farmers use mainly three varieties, ANAR 97, Altamira 9 and Altamira 14. In addition to increasing resistance to the aforementioned diseases, farmers are now asking for more early-flowering varieties in order to permit different planting and harvesting dates. The farmers appear to be satisfied with grain yield potential and grain quality of the above varieties.

In Masaya area, where favorable soils and climatic conditions allow diversified farming systems (including maize, rice, bean, cassava and fruits), rice is mainly produced for family consumption. Like in the San Juan Viejo area, the use of "official" modern varieties seems to be low. Since the 1990s farmers have been accessing new varieties and seed via informal exchange with other farmers or NGOs. The exact origin of these varieties is not known. Because of recent rainfall irregularity, farmers have been asking for more drought-resistant varieties with intermediate height (1-1.2 m) and resistance to lodging.

Sorghum

The diagnostic study carried out centered on four communities in the Madriz department. The study revealed a great diversity of sorghum cropping systems. Photoperiod-sensitive sorghum landraces, generally referred to as "millón" in Nicaragua and "maicillo" in Honduras, are planted in combination with maize, bean or insensitive sorghum with different geometric arrangements depending on soils, slopes or climatic constraints as well as farmers' strategies and goals. Because of its rusticity and ability to adapt to poor, unfertilized soils, as well as its very long cycle (may to December) which allow to support better middle season droughts, millón play an important role in the kind of low-risk crop that guarantees family subsistence production when maize and/or bean production has failed. Since the mid-1980s, short cycle and insensitive white grain sorghum have gradually been replacing maize and millon on the flattest and most fertile lands. As a result, millon sorghum is now more concentrated on the hillsides, as it is also the case in the area of Somotillo and in the semi-arid central hillside area of Ciudad Dario-Terra Bona. In the Madriz department exists an interesting connection between the diversity of sorghum varieties and the cropping systems with their diversity of production constraints and goals. Therefore, following the workshop organized in each of the communities, 30 varieties were collected in the four communities taking part in this study. Among them, 18 are supposedly photoperiodic-sensitive landraces and two are broomcorn varieties. All these varieties have been planted both on–farm and on the Managua CNIA station. At the on-farm site, a participatory characterization and classification of these varieties will be carried out with farmers from the four communities. More formal descriptions of these varieties will also be obtained from both sites. Numerous varieties are thought to come from the border countries of Honduras and El Salvador, demonstrating the importance of informal exchange of seed between farmers. No improved short-type millon varieties were found during this survey. Among short-cycle insensitive improved varieties, Tortillero precoz is the most largely diffused variety in entire Esteli region (Julio Molina, personal communication). This variety is a derived line from IRAT 204, a CIRAD/ISRA-improved variety from Senegal, which is well adapted to sahelian conditions and has a good grain quality. Short-cycle sorghum is mainly planted during the *postrera* season (August to November) in order to achieve three different objectives: grain for family consumption (tortilla, pinol), grain for chickens and pigs, and straw for ruminant alimentation (for farm needs or for selling). The importance of these objectives depends on production constraints and on the individual strategies of the farmers.

The following breeding objectives resulted from the different workshops organized with the farmer groups:

a) Photoperiod-Sensitive Sorghum:

- To decrease plant height in order to reduce risks of lodging and to better control head insects
- To improve grain yield
- To decrease plant cycle for harvesting in December (more early flowering and to reduce flowering-maturity duration)
- To improve straw quality (more green leaves at harvest, improved stem quality)

b) Short-Cycle Insensitive Sorghum:

- To improve grain and stover yields
- Improved resistance to bugs, sorghum midge and stocks insects
- Better grain size in order to achieve a better price
- Early cycle and drought resistance
- Grain quality to give good white tortillas
- To improve stover quality for ruminants

Crop Improvement

Following former participatory testing and selection with farmers initially carried out by the NGO Prodessa and then by CIAT hillsides project in collaboration with the CIALs in San Dionisio, IRAT 301 and IRAT 364/90 have already been adopted by farmers in the hillside central regions of Nicaragua. Data from on-farm trials along with qualitative information from farmers was collected in order to convince INTA to include these two varieties into the official validation for 2003 with a view to a possible official release in Nicaragua.

As a result of the last two years' on-farm trials with CIALs, the rice varieties IRAT 301, IRAT 362 and IRAT 364 are now in a phase of demonstration trials in the low hillside area of Yorito, Honduras. IRAT 364/90 and IRAT 392 are, meanwhile, in validation trials for high hillside conditions in the same region. An official release of the best two or three varieties has to be decided on in 2003.

For all on-station and on-farm trials conducted in 2002, the first results for participatory evaluation and selection with farmers groups and agronomic data will be available in October-November.

Futures Activities

1. Implementing activities of diagnostic and PVS trials at all sites in Nicaragua and Honduras.
2. Writing a project proposal to obtain funds from French Cooperation for Centro-America for further sorghum activities .
3. Visit to Guatemala and El Salvador to identify areas and possible partners and donors for the project (2002).
4. Visit to Haiti (2003).

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

3C. CIAT – FLAR Collaboration

The Latin American Fund for Irrigated Rice (FLAR), created in 1995, is one of the institutions located in Agronatura. CIAT, one of its founding partners, makes an annual contribution to be a member of FLAR. In addition, CIAT has provided financial and administrative support to FLAR as well as support for training. FLAR is mainly an outreach mechanism for scaling out research results generated at CIAT. The network of collaborators include nine country members with multiple organizations in each country linked to the private sector (producers, millers, seed multipliers) and the public sector.

As a service to FLAR, CIAT provides the infrastructure for the evaluation of rice lines for resistance to *T. orizicolus* and RHBV. CIAT also processes FLAR rice lines in the anther culture facility. The milling and cooking quality laboratory is a joint activity, with CIAT providing space and instruments and we share labor costs. FLAR maintains a germplasm bank, which contains well characterized materials for crosses. This has provided many of the materials that are going into the CIAT rice germplasm bank that will be maintained in the Genetic Resource Unit of CIAT.

This year, FLAR selected 81 advanced lines, mainly from the interspecific crosses made at CIAT, for further evaluation. Although germplasm exchange has mainly been by FLAR of CIAT lines, we expect that this will become a reciprocal activity. CIAT has free access to all FLAR lines for research purposes. FLAR, FEDEARROZ and the Rice Project have agreed to have closer collaboration in the development of improved germplasm with resistance to Rhizoctonia. Advanced lines derived from interspecific crosses with *O. rufipogon*, as well as Oryzica 3, Palmar, Pankai and Remadja will be used as donors. The CIAT rice pathology group and Patricia Guzmán of FEDEARROZ will do the greenhouse and field evaluation of the breeding populations. Some single crosses from FLAR that already had Palmar as a donor were top-crossed to Pankai, Remadja and will be one of the populations tested.

For rice blast resistance, the CIAT pathologist has collaborated with FLAR members in establishing a hot spot for rice blast in Brazil and training in breeding methodology for stable resistance. A long-term joint study between CIAT and FLAR was initiated in 2000 to evaluate the association of selection for blast resistance in early generations (F_2) and the stability of this resistance in advanced generations. Since blast resistance is the result of the action of many resistance genes, our hypothesis is that F_2 families which show a higher number of blast resistant plants, and which showed a higher number of resistant sister lines, will give origin to more stable resistant lines in the advanced generations. On the contrary, those lines originating from F_2 resistant plants selected within crosses where F_2 susceptible plants predominate, will be less stable. This study has implications for the method of selection for durable blast resistance and will help breeders to concentrate in those crosses with higher probabilities of yield stable blast resistant lines.

FLAR complements activities of CIAT in the area of crop management efforts. FLAR has ongoing crop management activities in Venezuela, Brazil, Colombia, Costa Rica, and Guatemala.

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

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Training

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18. Johanna Patricia Villamizar Ruíz. Undergraduate thesis. Identificación de cambios genéticos en el hongo *Pyricularia grisea* Sacc. Asociados a la pérdida de resistencia en líneas/variedades de arroz *Oryza sativa*. Universidad Nacional de Colombia Seccional Palmira. Mayo 2001-Octubre 2002. Presidente de Tesis Dr.Fernando Correa.

- **Workshops**

1. Reunión interna del Proyecto Arroz. CIAT. Noviembre 5-6, 2002. Presentaciones de trabajos realizados durante el año. Participants: 40.
2. Colombian Biosafety Workshop: Organized by OEA and Cambiotec, Canada. October 2002. Cartagena, Colombia. Z. Lentini Lecturer. Participants: 50 .
3. 2o. Taller Internacional sobre Manejo de Plagas en Arroz. Juma, Bonao, República Dominicana, Octubre 28 a Noviembre 1, 2002. Participants: 40
4. Biosafety Workshop: Z. Lentini, P. Chavarriaga, J. Tohme. Coordination of Workshop on Agriculture Biosafety for the Colombian Ministry of Environment. September 15-18. CIAT, Cali, Colombia. Participants: 25.

5. Valès Michel. Taller II, "Desarrollo participativo del arroz de laderas" Escuela Verde, Santander de Quilichao, Cauca, Agricultores pertenecientes a la red ARDECAN de CETEC. Abril 12, 2002. Participants: 13.
6. International Course on Biosafety of GMOs: Organized by University of United Nations, Tokyo Chapter BioLAC. April 2002. Caracas, Venezuela. Z. Lentini Lecturer. Participants: 30.
7. II Taller Internacional de Selección de Arroz de Secano. Bolivia-Santa Cruz de la Sierra, March 4-9, 2002.
8. Châtel, M.; Ospina, Y.; Gamboa,C.; Marassi, M.A.; Marassi, J.E.; Hernaiz, S.; Alvarado, R., Taboada, R.; Guzmán, R.; Pérez, R. Composite population breeding using recurrent selection in Latin America and the Caribbean. 29th Rice Technical Working Group Meeting. Arkansas, U.S.A. February, 2002. Participants: 300.
9. Biosafety Open House: Z. Lentini, P. Chavarriaga, J. Tohme. Workshop on the Biosafety of GMOs for the CIAT community. November 27 – December 2, 2001. Participants: 100.
10. I Taller Internacional de Selección Recurrente de Arroz de Riego.Venezuela-San Felipe, October 2001. CD ROM publication by Fundación DANAC-Venezuela, CIAT/CIRAD-Colombia and EMBRAPA-Brazil. Participants: 31.
11. II Taller Internacional de Arroz de Secano en Bolivia. Marzo 4-9, 2002. Moreno Berrocal, A.; Châtel, M.; Guimarães, E.; Ospina,Y. Estudio agronómico del sistema arroz (*Oyza sativa* L.) intercalado con siembras nuevas de café (*Coffea arabica* L.). Participants: 18.

- **Formation and Training**

1. José Martinez Teruel, student at the ENITA of Clermont-Ferrand, France. He was received by Fundación DANAC-Venezuela to do its technical practice work, during 6 months from May to October 2002. Supervisor Dr.Marc Châtel.
2. José Hurtado. INIA. Programa de Arroz del Ecuador. Entrenamiento en Mejoramiento de Arroz bajo la supervision del Dr.Martínez durante Junio y Julio 2002 en CIAT Palmira y en Laboratorio de Fitopatología y entrenamiento de campo en la Estación Experimental de Santa Rosa bajo la supervision del Dr.Correa en Agosto de 2002.
3. Renata Pereira da Cruz. Ph.D. IRGA Brasil. Cultivo de Anteras. 12 Agosto, 2002. Supervisor Dr.Zaida Lentini.
4. Jaime Arias. Pasantía financiamiento Cenagref. Estudio de nichos de mercado del arroz de pequeños productores de zonas marginales. Julio de 2002 a Marzo de 2003. Supervisor Dr.Michel Valès.
5. Fabiana Malacarne, Ph.D. Genetic transformation, field testing, biosafety, gene flow. Universidad Central de Venezuela, sede Maracay. 15 April-15 May, 2002. Supervisor Dr.Zaida Lentini.
6. Sandra Milena García Barrero. Pasantía 4 meses. Estudiante de Bacteriología. Universidad Católica de Manizales. Marzo-Junio, 2002. Supervisor Dr.Fernando Correa.

7. Tania Quesada, M.Sc. Training on microsatellite analysis of rice and their use for gene flow analysis into wild/ weedy relatives. University of Costa Rica. 10-23 March, 2002. Supervisor Dr.Zaida Lentini.
8. Griselda Arrieta, M.Sc. Training on rice crossing, transgenic field testing, biosafety, and RHBV evaluation. University of Costa Rica. 10-23 March, 2002. Supervisor Dr.Zaida Lentini.
9. Erika Arnao, M.Sc. Microsatellite for rice characterization. Universidad Central de Venezuela, sede Maracay. Supervisor Dr.Zaida Lentini.
10. Renata Pereira da Cruz. Ph.D. IRGA Brasil. Cultivo de Anteras. Supervisor Dr.Zaida Lentini. 12 Agosto, 2002.

Information

- **Refereed Publications**

1. Lentini Z., Lozano I, Tabares E., Fory L., Domínguez J., Cuervo M., Calvert L. 2002. Expression and inheritance of hypersensitive resistance to rice hoja blanca virus mediated by the viral nucleocapsid protein gene in transgenic rice. Theoretical and Applied Genetics (*B1415, In Press*).
2. J.L. Fuentes, F.J. Correa-Victoria, F. Escobar, L. Mora, M.C. Duque, J.E. Deus, y M.T. Cornide. Análisis de la Diversidad Genética de Poblaciones del Patógeno del Añublo del Arroz en dos Localidades en Cuba. Revista: Biotecnología Aplicada. Aceptado para publicación en Octubre 22 de 2002.
3. Nguyen, VT, Bay D. Nguyen, Surapong Sarkarung, Cesar Martinez, Andrew H Paterson, Henry T Nguyen. Maping genes controlling Al tolerance in rice :Comparing different genetic backgrounds.2002. Molecular Genetics and Genomics.Published on line June 07/02.
4. Thomson M.J., Tai T.H., McClung A.C., Hinga M.H., Lobos K.B., Xu Y., Martínez C., McCouch S.R. 2002. Mapping quantitative trait loci for yield components, and morphological traits in an advanced backcross population between *Oryza rufipogon* and the *Oryza sativa* Jefferson. Submitted to Theoretical and Applied Genetics.

- **Book Chapters**

1. Châtel, M. 2001. Rice Improvement Using Conventional Breeding and Gene Pools and Populations with Recessive Male-Sterile Genes. Annual Report CIRAD/CIAT. CIRAD/CIAT Document. October 2001.
2. Lentini, Z. 2002. Unique Challenges and Opportunities for Environmental Assessment of GMOs in the Tropics. *In*: GMOs and the Environment. OECD. Raleigh-Durham, the United States *(In Press).*
3. Lentini, Z. 2002. Gene Flow Analysis for Assessing the Safety of GMOs in the Neo-Tropics: The case of beans and rice. *In:* GMOs: Real Risks or Chimeras? UNIDO, Austria *(In Press)*.

4. Trouche Gilles. (In press). 2003 El mejoramiento poblacional participativo y descentralizado de Arroz. Progresos en el Mejoramiento Poblacional en Arroz. E.P.Guimarães (Ed.).

- **Other Publications**

1. Hernaiz, L. S.; Alvarado, A. R.; Châtel, M.; Borrero, J. (2001). Creación de la población PQUI-1 desarrollada con tolerancia al frío mediante selección recurrente en arroz. Agricultura técnica (Chile) 60:195-199.
2. vom Brocke, K.; Trouche, G. and C. Barro. Evaluation of data from participatory selection in segregating material of sorghum in two villages in Burkina Faso. Paper presented in the workshop of quality of science in participatory plant breeding, September 30-October 4, Roma, Italy.
3. vom Brocke, K.; Trouche, G.; Vaksmann, M. and Bazile, D. 2002. Préservation de l'agrobiodiversité du sorgho au Mali et au Burkina – amélioration de la productivité et maintien de la biodiversité. In : Proceedings of the « Atelier sur la Conservation et valorisation des ressources génétiques des mils », May 28-29 2002, Niamey, Niger. In press.
4. Dakouo, D. ; Trouche, G. ; Bâ, Malick and Zongo Adama. 2002. Perspectives de lutte contre la cécidomyie du sorgho, *Stenodiplosis sorghicola*, une contrainte majeure à la production du sorgho dans les zones Centre Ouest et Est du Burkina Faso. In : Proceedings of the « Forum de la recherche scientifique et de l'innovation technologique, April 2002, Ouagadougou, Burkina Faso. In press.
5. Ratnadass, A.; Trouche, G.; Chantereau, J.; Dakouo, D.; Ag Hamada, M; Fliedel, G. and Luce C. 2002· Selection of a sorghum line CIRAD 441 combining productivity and resistance to midge and head-bugs. International Sorghum and Millet Newsletter vol 42. In press.
6. Hocdé, H. ; Lançon, J. and G. Trouche 2002 (eds) 2002. La sélection participative: Impliquer les utilisateurs en amélioration des plantes. Proceedings of a workshop held in Montpellier, May 5-6, Montpellier, France, 148 p. CIRAD working document on line www.cirad.fr.
7. Hernaiz, L. S.; Alvarado, A. R.; Châtel, M.; Ospina, R. Y. *(To be published at the end of 2002).* Creación de la población PQUI-2 desarrollada con tolerancia al frío para selección recurrente en arroz. Agricultura técnica (Chile).
8. Marassi, M. A.; Marassi, J. E.; Châtel, M.; Borrero, J. Avances en el desarrollo de poblaciones de arroz en Argentina. Published May 2002 in the electronic review: e-campo.com. http://www.e-campo.com.
9. Châtel, M.; Ospina, Y. Mejoramiento genético avanzado de arroz. Mejoramiento poblacional con androesterilidad genética recesiva. INIA Quilamapu-Chile, March 8, 2002.
10. Châtel, M. Los Recursos Genéticos deArroz del CIRAD. Mejoramiento convencional. Impacto en America Latina. II Taller internacional de arroz de secano. CIAT Santa Cruz, March 4-9, 2002.

11. Moreno B., A. M.; Châtel, M. El Arroz de Secano, una Nueva Opción para la Diversificación de la Agricultura en la Región Cafetera de Colombia. II Taller internacional de arroz de secano. CIAT Santa Cruz, March 4-9, 2002.
12. Ospina,Y.; Châtel, M.; Rodriguez, F.; Lozano, V.H. El Mejoramiento Poblacional del Arroz de Secano para las Sabanas Colombianas. II Taller internacional de arroz de secano. CIAT Santa Cruz, March 4-9, 2002.
13. Fernando Correa-Victoria, Didier Tarreau, Cesar Martinez, Fabio Escobar, Gustavo Prado, Girlena Aricapa. 2002. Studies on the rice blast pathogen, resistance genes, and implications for breeding for durable blast resistance in Colombia. Abstracts.3rd International Rice Blast Conference. 11-14 Septiembre 2002. Tsukuba, Japan p64.
14. C.P.Martinez, P.Moncada, J.Lopez, A,Almeida, G.Gallego, J.Borrero, MCDuque, F.Correa, C.Bruzzone and J,Tohme. 2002. Utilization of new alleles from wild rice species to improve cultivated rice in Latin America. Abstracts International Rice Conference.16-20 September 2002,Beijing, China. p271.
15. M.J,Thomson, E.M. Septiningsih, K.R. Trijatmiko, J.Alcala, C.Martinez, A.McClung, S. Moelpojawiro, and S.R.McCouch.2002. Targeting functional nucleotide polymorphisms (FNPs) underlying QTL introgressed from O.*rufipogon*. Abstracts International Rice Conference 16-20 September 2002,Beijing, China. p71.
16. Châtel, M.; Ospina, Y.; Rodriguez, F.; Guimarães, E.P.; and LAC Cooperators.
17. Los Recursos Genéticos para el Mejoramiento Poblacional del Arroz. Proyecto Colaborativo CIRAD/CIAT/LAC NARS. II Encuentro Internacional de arroz. La Habana-Cuba, July 10-12, 2002.
18. Guimarães, E.; Châtel, M. Enhancing Genetic Diversity in Rice Production in Latin America through Population Improvement. International Rice Commission (IRC) Meeting held in Bangkok, Thailand, July 23-25, 2002.
19. Châtel, M.; Mendez del Villar P.; Ferreira, C.M.; de Raissac, M. Perspectivas del sector arrocero en América Latina. Primero Congreso da Cadiea Produtiva de Arroz. VII Reunião de Pesquisa de Arroz-RENAPA. Florianópolis-Brazil. August 20-23, 2002.
20. Valès Michel. "Selección recurrente y mejoramiento participativo del arroz de secano con tolerancia al frio para pequeños productores de las cordilleras colombianas" Junio 5 /02. Seminario interno y extrerno CIAT. Se invitaron 14 personas socios del trabajo con los agricultures.
21. F. Correa-Victoria, D. Tharreau, C. Martinez, M. Vales, F. Escobar, G. Prado, and G. Aricapa. Identification of gene combinations for developing durable rice blast resistance in Colombia. International Rice Congress. 16-20 September 2002, Beijing, china.
22. F. Correa-Victoria, D. Tharreau, C. Martinez, F. Escobar, G. Prado, and G. Aricapa. Studies on the rice blast pathogen, resistance genes, and implications for breeding for durable blast resistance in Colombia. 3rd. International Rice Blast Conference. 11-14 September, 2002. Tsukuba, Japan.
23. Fernando Correa. Identificacion de combinaciones de genes en arroz para el desarrollo de resistencia durable a Pyricularia grisea. 2do. Encuentro Internacional de Arroz. 10-12 de julio de 2002, La Habana, cuba.

24. P. Ruíz, J.J. Vasquez, E. Corredor, E.Gonzalez, L.F. Fory, A. Mora, J. Silva, M.C. Duque, and Z.Lentini. 2002. Poster No. 14, page. 287. *In:* The 7th International Symposium on the Biosafety of Genetically Modified Organisms. Beijing, China. October 10-16, 2002.
25. E. Gonzalez, L.F. Fory, J.J. Vasquez, P. Ruíz, E. Corredor, A. Mora, J. Silva, M.C. Duque, and Z.Lentini. 2002. Poster 16, page. 288. *In:* The 7th International Symposium on the Biosafety of Genetically Modified Organisms. Beijing, China. October 10-16, 2002.
26. Z. Lentini, E. Tabares, L. Fory, A. Mora, E. Gonzalez, J.J. Vasquez, P. Ruíz. 2002. Expression and Inheritance of RHBV in Transgenic Rice Breeding and Biosafety in the Neo-Tropics. Invited Key Note Speaker. RicEU Conference. Torino, Italy, June 6-8, 2002.
27. C.P.Martinez, P.Moncada, J.Lopez, A.Almeida, G.Gallego, J.Borrero, M.C.Duque, F. Correa, C.Bruzzone, J.Tohme, and Z.Lentini. 2002. Gene Technology: Expanding Genetic Diversity and Adding Value to rice. Invited Key Note Speaker. RicEU Conference. Torino, Italy, June 6-8, 2002.
28. Hurtado M. Elizabeth. Recopilación Bibliográfica Investigadores del Proyecto de Arroz del CIAT, 1971-2002. Centro Internacional de Agricultura Tropical (CIAT). Cali, CO. 336p.
29. Calvert, L.A., Reyes, L., Meneses, R., Cruz, M, and Triana, M. Desarrollo de resistencia varietal al Virus de la Hoja Blanca de arroz en Colombia. Presentation for Socolem July 2002 Short Article

- **IP-4 Web Site** – María Nelly Medina

Starting in May 2002 CIAT launched a new format for its Web Site. This format is more user friendly and contains much more information that is useful to our partners than the previous version. The CIAT Rice Project Web Site is in both English and Spanish which fulfills the needs of the majority of our visitors. Posters, pamphlets, and manuals of selected research topics are included to highlight strategic work and give greater access to this information. Significant effort is also devoted to keeping the site updated with relevant information on contacts, publications, research activities at CIAT as well as events in the rice sector. We hope that you find the CIAT Rice Project Web Site useful.

http://www.ciat.cgiar.org/riceweb/index1.htm
http://www.ciat.cgiar.org/riceweb/esp/inicio.htm

Annex 1. Principal and Support Staff

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Fernando Correa, *Plant Pathologist*
Zaida Lentini, *Plant Breeder*
Mathias Lorieux *Molecular Biologist*
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Rafael Meneses, *Entomologist, I.I.A., Cuba*
Gilles Trouche, *Plant Breeder, CIRAD-CA*
Michel Valès, *Plant Pathologist, CIRAD-CA*
Carlos Bruzzone, *Consultant Peru*

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- **Technicians and Field Support**

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