Annual Report 2001

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

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CIAT
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Project IP-4 Improved Rice Germplasm for Latin America and the Caribbean Annual Report 2001 Centro Internacional de Agricultura Tropical (CIAT) Cali Colombia October 2001

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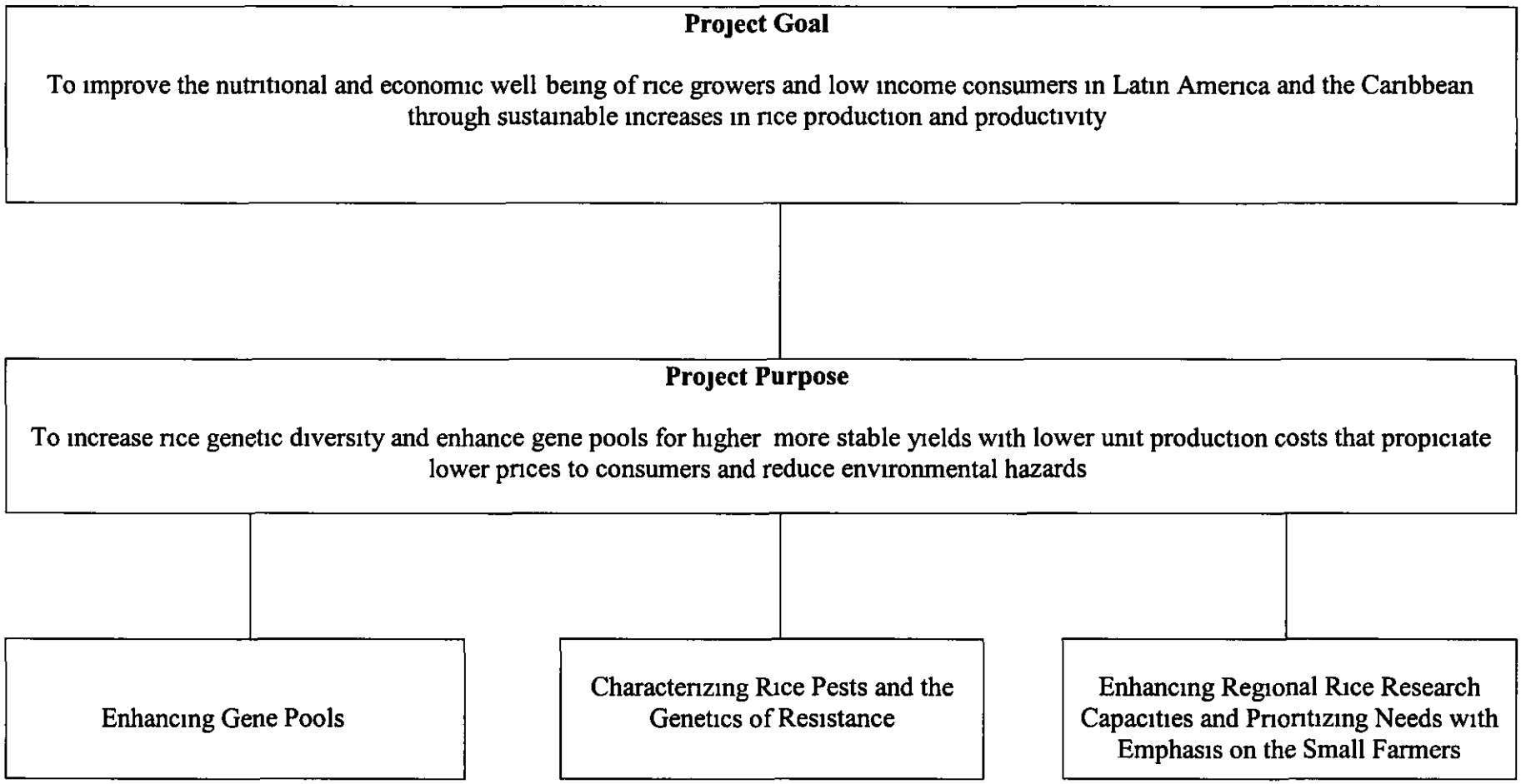

Project Log Frame

Narrative Summary	Measurable Indicators	Means of Verification	IMPORTANT ASSUMPTION
Goal Germplasm of beans cassava tropical forages rice and their wild relatives collected, conserved and enhanced and made accessible to NARS and other partners	A sufficient number of accessions (of beans cassava and tropical forages) representing genetic diversity are conserved and managed ex situ Strategies and guidelines for in situ management of biodiversity of beans cassava and tropical forages have been developed and tested with users Accessible germplasm of beans cassava tropical forages and rice meet NARS standards in terms of productivity stability agronomic traits and user needs Techniques and relevant information for more efficient and reliable germplasm improvement are accessible to users	CIAT s germplasm bank inventories Partners technical reports Annual reports	
Purpose To increase rice genetic diversity and enhance gene pools for higher more stable yields with lower unit production costs that propiciate lower prices to consumers and reduce environmental hazards	Evaluations of yield potential (interspecific wide elite crosses and recurrent selection) Continued use of improved germplasm by NARS • Monitoring rice production practices and markets IPM practices in place for stable production and cleaner environment Rice lines selected with desired gene traits Potential sources for high levels of biotic and abiotic stress resistance	Databases • Project, CIAT and NARS annual reports Publications Promotional Activities (conferences training workshops field days)	Stability (internal and external) National policies favor adoption of i technology
Outputs 1 Enhancing Gene Pools 2 *Characterizing* Rice Pests and the Genetics of Resistance 3 Enhancing Regional Rice Research Capacities and Prioritizing Needs with Emphasis on the Small Farmers	Pathogen/pest variation and source of resistance identified IPM strategies Workshops Training courses Farmers surveys	Project progress report for 2000 Publications Project progress and workshop reports	Continued support from CIAT/CIRAD/FLAR Continued adequate funding Recommendations adopted by NARS an implemented by farmers

NARRATIVE SUMMARY	MEASURABLE INDICATORS	MEANS OF VERIFICATION	IMPORTANT ASSUMPTION
OUTPUT 1 ENHANCING GENE POOLS **Activities** A Rice improvement using conventional breeding and gene pools/populations with recesive male sterile genes Evaluation of savannas upland rice lines in Latin American countries B Developing upland rice for small landholders C Advance and evaluation of inter specific gene pools D Introgression of new plant type genes into LAC s gene pools E The use of anther culture and in vitro culture for enhancement of gene pools	• Rice populations developed and improved (tolerance soil acidity resistance to blast, RHBV *T orizicolus* (13) good grain quality early maturity Number of field trials planted and lines selected Populations distributed to NARS for line development Populations developed (14) populations in process (12) populations yield tested/molecular characterized (4) Partners (WARDA CIRAD EMBRAPA CORNELL) Number of crosses made (433) tropical irrigated (226) temperate (155) upland (52) Number of selected lines Double haploids interspecific crosses (386) acceleration breeding populations (815) somaclones (3758 Venezuela 4440 Colombia)	Project progress report for 2001 Field visits and evaluations in testing sites Breeding populations distributed to LAC Breeding populations in storage and field Best lines and QTL S identified Breeding populations in storage and field Double haploids in storage Publications	Continued support from CIAT/CIRAD/FLAR Adequate funding and timely release budget • Favorable climate Continued financial support for anth culture lab Crosses field support and operation costs provided by FLAR

Narrative Summary	Measurable Indicators	Means of Verification	IMPORTANT ASSUMPTION
OUTPUT 2 CHARACTERIZING RICE PESTS AND THE GENETICS OF RESISTANCE **Activities** A Characterizing the interactions of host plant resistance to rice blast, sheath blight and grain discoloration B Characterizing and using partial and complete resistance for the control of rice blast C Characterizing the interactions of host plant rice hoja blanca virus and *T orizicolus* complex D Foreign genes as novel sources of resistance to rice hoja blanca virus and *Rhizoctonia solani* E Characterizing the interactions of host plant, *Polymyxa graminis* and rice stripe necrotic virus that causes entorchamiento	• Virulence spectrum and genetic structure of rice pathogens • Molecular markers associated and number of resistance genes Sources of complete complementary and partial resistance Rice lines with diversified resistance to RHBV and *T orizicolus* Understanding components of resistance to the RHBV complex *Crop management components* developed • Transgenic lines with RHBV viral genes with reduced symptoms produced and evaluated • Transgenes introgressed into commercial cultivars Using novel genes for multicomponent resistance to rice pathogens Characterization of the RSNV and vector complex Development of germplasm evaluation methods	• Pest and disease resistant varieties released by partners • *Collection of rice pathogens* Database of resistance sources Crosses made among resistance sources • F7 lines with stable blast resistance combining genes Pi 1 and Pi 2 • Rice genome map with blast resistance genes mapped Rice progress report for 2001 Publications Resistant germplasm selected under artificial conditions	Rice crosses and populations develo by breeders Biotech Unit identify molecular ma associated with resistance • Continue collaboration with FLAR Continue adequate funding from Colombia and Rockefeller • Continue support and adequate fund from CIAT CIRAD and FLAR Continued funding from Colombia Rockefeller Colciencias • Permission for field testing of transg plants is granted • Continued support and adequate fun

OUTPUT 3 ENHANCING REGIONAL RICE RESEARCH CAPACITIES AND PRIORITIZING NEEDS WITH EMPHASIS ON THE SMALL FARMERS **Activities** A Participatory Development of of Rice for Poor Communities in Marginal Areas B FLAR – CIAT collaboration C Collaborators Training and information	Community based projects New varieties of small equipment for rice systems Number of scientists trained Workshops Published reports of courses FLAR annual report • Publications Web pages	• Increased production in marginal areas No of communities participating Rice progress report for 2001 CIAT s Web page	• Special funds continue • Recommendations adopted by farme

Introduction

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

But will this tremendous increase in our knowledge of rice meet the challenge of making these varieties available to the resource poor farmer? It will not unless we understand the needs of these farmers This is another area where the CIAT rice project is striving to increase our efforts By using methods such as participatory selection of varieties and surveys to find out what are the regional preferences we are gaining more insight into the needs of the resource poor rice farmer Working with small farmers in the Pacific coast of Colombia and in the mid altitude regions of the Andes we are increasing our understanding of their needs and the many challenges that need to be addressed if we are going to successfully meet their needs

The Following Highlights Illustrate a part the Strategy and Successes of the CIAT Rice Project

New Varieties for Colombia

In the last three years FEDEARROZ has released 5 new varieties for Colombia FEDEARROZ and CIAT have worked closely together in the development of these varieties Traits including resistance to rice blast and rice hoja blanca virus and its vector as well as quality analysis were areas of collaboration The success of Fedearroz 50 has surpassed expectations Released late in 1998 more than 50% of the rice grown in Colombia is now Fedearroz 50 This variety is yielding on average 7 t/ha and this is the principal reason that Colombia returned to self sufficiency in rice production after several years of deficits More recently Fedearroz 2000 Victoria 1 Victoria 2 and Colombia XXI were released Several of these promise to become important varieties but they must compete with Fedearroz 50 CIAT will continue to work with FEDEARROZ and other national partners to have impact throughout the region

Rice and its Relatives

There is relatively little genetic diversity in cultivated rice in Latin America The narrow base of the commercial varieties makes them susceptible to both biotic and abiotic stresses Interspecific crosses with *O rufipogon* are being used as a source aluminum tolerance and root growth Advanced lines from the cross Bg90 2/*O glaberrima* were identified as resistant to crinkling disease Advanced lines from the cross Lemont/*O barthii* mature early with good yields and excellent grain quality Wild relatives are contributing to increasing the nutritional content of iron zinc and protein of rice There are many sources of resistance to pests diseases and to abiotic stress factors These are just a few examples of the traits that are being incorporated into rice using genes from its wild relatives Interspecific crosses also increase the genetic diversity of the crop The diversity of the parents makes these interspecific crosses ideal in studies to develop molecular markers Already they have been used to mark regions of the chromosome of rice for important traits Varieties with more diversity that yield well in environments with low inputs and are more nutritional may sound too good to be true yet they are our goal and the future of rice

Taking Aim at a Moving Target Rice Blast

Most commercial varieties remain resistant to rice blast for only one to three years There is a tug of war between the pathogen and the host While a host may be resistant to many of the rice blast isolates there always seem to be a subset of the population that is not recognized by the plant s defenses and it soon becomes the predominant When this happens the resistance is broken and the new variety becomes susceptible to rice blast Hot spot selection under high disease pressure and pathogen diversity has been the principal method for breeding rice blast resistant lines and varieties Obviously something more is needed Oryzica Llanos 5 is a variety that was developed through hot spot breeding and is exceptional because it has remained resistant to rice blast for more than a decade We are dissecting the genome of Oryzica Llanos 5 to identify its combination of resistance genes And this is part of a larger effort to catalog both the resistance genes in the plant as well as the virulence genes in the fungus Using near isogenic lines carrying individual resistant genes and biological testing with the known rice blast linages progress is being made The search is on for molecular markers and with the information from the rice genomic sequencing project the rate of discovery of rice blast resistant genes is increasing This will facilitate the isolation characterization and utilization of these genes Already we are testing associations of these genes in order to develop rice varieties that have a series of resistant gene combinations that confer durable resistance

Rice for the Small Resource Poor Small Landholders

Impact assessment has shown that both large and small farmers benefit equally from new technologies when they are in the same agroecosystem In the Latin America and Caribbean region most rice producers are small farmers living in marginal areas and they have not benefited from the technologies that are improving production in major rice growing zones The CIAT rice project is increasing our efforts to reach these resource poor rice farmers Very high rainfall agricultural systems have unique problems These include flooding low luminosity high disease incidence as well as post harvest problems including the drying of the grain Other marginal environments are in the moderate rainfall hillsides In these environments drought pests and diseases are all major problems Yields and grain quality are important to both the producers and consumers Several pilot projects have been started in Colombia Breeding populations have begun for many of these difficult environments Next year with the collaboration of CIRAD we will add a participatory rice and sorghum breeder in Central America We are being practical and developing gardens of varieties for the first rounds of selection and this should have impact very soon Then we must develop truly superior rice lines adapted to the region developed together with the small farmers With this strategy we hope to insure food security and better economic viability to the poorest of the poor

Future Perspectives

The rice project has the challenge of being part of the bridge between the rapid advances in the molecular characterization of rice and producing usable products for the rice farmers Rice will be the first major crop for which the complete genomic sequence will be available The Rice project (IP 4) and Biotechnology (SB 2) are working together to develop the strategic alliances in the area of rice genomics The Rice project is also strengthening partnerships to better meet the needs of rice farmers with emphasis on the resource poor small farmer To make these partnerships work we depend on support both from the region and the developed countries This strategy is partially in place and we wish to gratefully acknowledge the support of the Colombian Ministry of Agriculture CIRAD and the government of France Rice is the most important food crop in the world and we are dedicated to helping provide the technologies to assure food security and helping the rice farmers

OUTPUT 1 ENHANCING GENE POOLS

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

- **Upland Savannas Ecosystem**

Conventional Crossbreeding

M Chatel Y Ospina F Rodriguez and V H Lozano

Abstract

Conventional crossbreeding has permitted the development and release of modern upland lines in Latin America (Bolivia Brazil and Colombia) CIAT and CIAT/CIRAD projects now concentrate on broadening the genetic base of upland rice Inter specific crosses between *Oryza sativa* and the African cultivated *Oryza Glaberrima* and wild relative species is one of the new breeding strategies used to achieve this objective Since 1996 no new conventional *Japonica Oryza sativa* by *Japonica Oryza sativa* crosses were made Though as we have advanced segregating lines in the pipeline we continue their evaluation and multiplication in Colombia for release to Latin American and Caribbean (LAC) NARS partners

Key words Conventional crossbreeding *Japonica Oryza sativa* Latin America, line release genetic base

Introduction

CIAT and CIAT/CIRAD projects are phasing out *Oryza sativa* subsp Japonica by *O sativa* subsp Japonica conventional crossbreeding activities and concentrate on broadening the genetic base of upland rice (Cuevas Perez et al 1992) Inter specific crosses of *O sativa* by *O glaberrima* and *O sativa* by wild relative species is a new breeding strategy to achieve this objective

Since 1996 no new conventional *Japonica Oryza sativa by Japonica Oryza sativa* crosses were made for the development of segregating and fixed lines Though as we have advanced segregating lines in the pipeline we continue their evaluation and multiplication in Colombia for release to Latin American and Caribbean (LAC) NARS partners

We also evaluate and select NERICA lines (NEw RICes for Africa) introduced from WARDA and inter specific progenies from crosses between *Oryza sativa* and wild rice species developed at CIAT

Advanced upland lines selected in Colombia or introduced from CIRAD were shipped to regional NARS for evaluation use in breeding programs and possible release

Materials and Methods

Sources of rice lines

During the cropping season 2001 a total of 1613 lines were evaluated at La Libertad Experiment Station (LES) Villavicencio – Meta These included 694 traditional intra specific (*O sativa*) lines that were evaluated the seed was increased and shipped to NARS for use in their breeding activities Nine NERICA (*O sativa* subsp Japonica with *O glaberrima*) lines that had been introduced from WARD were evaluated Also 910 inter specific progenies that were developed at CIAT including crosses of *O sativa* by *O Glaberrima* (488 lines) and *Oryza sativa* by *O barthii* (422 lines) were evaluated

Experimental design

Each line was sown in plots of two rows that were 5m long with 0 26 m between rows For each 23 lines there were three checks plots consisting of Oryzica Sabana 6 Oryzica Sabana 10 and Linea 30 (CIRAD 409) The initial vigor tolerance to soil acidity and diseases plant height days to flowering and grain shape were rated

Results and Discussion

Traditional intra specific (*O sativa*) crossbreeding

These lines were advanced upland lines selected in Colombia or introduced from the rice genetic resource core collection of CIRAD Each evaluated line was selected and bulk harvested separately Samples of fresh seed were stored at CIAT and also were shipped to NARS for local evaluation This is part of the conventional breeding program and these lines were evaluated and selected by regional Latin American (Bolivia Brazil Colombia) and Asian (China and Vietnam) NARS This collaborative effort has been fruitful as demonstrated by a summary of the released varieties

Latin America

Bolivia

In 1999 the line CIRAD 170 was released as JASAYE for upland small holders The Bolivian Center for Research (CIAT Santa Cruz) and the Japanese Cooperation (JICA) are promoting the use of the line through demonstration plots seed multiplication and diffusion

Brazil

The CIAT/CIRAD materials continue to be selected and used in Brazil The major characteristics that were selected by the Brazilian scientists included earliness plant and grain type During the period 1994 1999 from a total of 6 varieties were released in the Brazilian States of Goias

Maranhao Mato Grosso Mato Grosso do Sul Minas Gerais Para Piaui Rondonia Roraima and Tocantins and four of these were from the CIAT/CIRAD project

CANASTRA	CT7415 6 5 1 2 B
MARAVILHA	CT6516 23 10 1 2 2 B
BONANÇA	CT11614 1 4 1 M
CARISMA	CT11251 7 2 M M

Colombia

Linea 30 or CIRAD 409 has already been tested in different cropping systems in rotation with others crops and in inter cropping with perennial species The line shows adaptation to acid soils earliness good yielding potential and grain quality We are working with CORPOICA Regional 8 of Villavicencio to get the line officially released as a variety

Asia

China

CIRAD CA has close links with the Food Crops Research Institute (FCRI) of the Yunnan Academy of Agricultural Sciences (YAAS) and had shipped CIAT/CIRAD and CIRAD (IRAT) upland lines in the recent years After screening they were released directly or used as parents In 1996 and 1998 IRAT104 and IRAT359 were released in the Yunnan Province In 1999 FCRI/YAAS released the variety YUNLU29 coming from a cross between a Chinese line and IRAT216

Vietnam

Two CIRAD lines (IRAT 177 and IRAT 216) were registered without modification as cultivars Of the 10 most promising upland/hillsides lines 5 are from the CIAT/CIRAD project and have CIRAD (IRAT) parents

CT6516 24 3 2 Two out of three parents are IRAT121 and IRAT216
CT10575 5 5 3 M 2 1 M One out of seven parents is IRAT 216
CT10576 21 4 M 1 3 M Two out of seven parents are IRAT124 and IRAT216
CT11620 29 2 M 4 M M Three out of nine parents are IRAT122 IRAT124 and IRAT216
CT11626 2 5 M M Three out of nine parents are IRAT124 IRAT194 are IRAT 216

It is interesting to note that line CT6516 24 3 2 comes from the same original cross as MARAVILHA (CT6516 23 10 1 2 2 B) released in Brazil

Interspecific line evaluation

NERICA lines introduced from WARDA

From the 9 lines evaluated 2 were selected and harvested for new evaluation to be done next year

Inter specific progenies developed at CIAT

From the 910 lines evaluated 69 were selected and harvested for new evaluation to be done nest year The results of evaluation and selection were sent to Dr Cesar Martinez who developed this germplasm

Upland Line Registration

If a specific line does well in a given country the national research institution may decide following the local legislation to name it and recommend its commercial cultivation acknowledging in the application form the genetic and institutional intellectual property CIRAD has a mechanism by which breeders can register specific material not necessarily release as variety It receives a consecutive CIRAD catalogue number and the country name where it was bred (see ref 3) If the line was release with a local name for commercial cultivation it is also mentioned

In 2001 we applied for the registration of 1 upland savanna line CT 10069 27 3 1 4 that performed very well in the Colombian Andes at mid altitude (1400 m a s l) when grown in association with young coffee plantations (grain yield of more than 5 T/ha without affecting further coffee production)

Conclusion

Conventional upland rice cross breeding have permitted the release of enhanced lines in different Latin American countries as well as in Asia
However the released lines are of narrow genetic base and there is a need for broadening it using new breeding strategies

Since 1996 following CIAT s recommendations the CIAT rice project IP-4 and the CIAT/CIRAD collaborative project are phasing out *Japonica O sativa* by *Japonica O sativa* conventional cross breeding activities and concentrates on broadening the genetic base of upland rice

Inter specific crosses between *O sativa* and wild relative species (CIAT project IP 4) as well as population breeding (CIAT/CIRAD project) are new breeding strategies to achieve this objective

The number of progenies developed from population breeding has steadily increased from 1997 on In 2001 more than 90% of the lines under evaluation and selection come from recurrent populations

Future activities

1 Seed multiplication of selected upland lines and dispatch to NARS for evaluation and selection
2 Long term conservation at CIAT germplasm bank

References

1 Cuevas Perez F E Guimaraes E P Berrio L E Gonzalez D 1992 Genetic base of irrigated rice in Latin America and the Caribbean 1971 to 1989 Crop Sci 32 1054 1059
2 Catalogue CIRAD des resources genetiques riz CIRAD Publication

- **Composite Population Breeding**

M Chatel Y Ospina F Rodriguez and V H Lozano

Abstract

Upland rice populations with a broad genetic base were developed and enhanced through recurrent selection breeding strategies This breeding strategy used a recessive male sterile gene (ms) from a mutant of IR36 to facilitate the development of these rice populations Site specific populations were developed in collaboration with NARS In Colombia upland populations were enhanced using two recurrent selection breeding methods At each enhancement cycle fertile plants are selected In 2001 more than 90% of the lines under evaluation and selection came from recurrent populations The most advanced lines were evaluated in yield trials in Colombia and many lines are very promising

Key words Rice populations genetic base male sterile gene enhancement recurrent selection and promising lines

Introduction

Although conventional upland rice cross breeding has been very successful in the development of new varieties in Latin American and Asia the released lines have a narrow genetic base and there is a need for broadening it Using new breeding strategies best does this The recessive male sterile gene (ms) from a mutant of IR36 rice population development was eased Basic populations were enhanced using two recurrent selection breeding methods From the basic germplasm and at each enhancement cycle fertile plants are selected and are the starting point for the generation of segregating progenies These are then evaluated and selected by conventional pedigree method The number of progenies developed from population breeding has steadily increased from 1997 and by 2001 more than 90% of the lines under evaluation were from recurrent populations

We shipped basic and enhanced populations as well as segregating lines to regional Latin American (Bolivia Brazil Colombia) and Asian (China) NARS for evaluation and selection

Site specific populations were developed with partners The most advanced lines were evaluated in yield trials in Colombia and the results in terms of yield and disease resistance are very promising

Materials and Methods

Source of rice germplasm

The upland rice population breeding activities aims at developing adapting and improving tropical *Japonica* *Oryza sativa* populations

The first source of these upland and lowland rice populations were created in the framework of a collaborative project (1984 1991) between EMBRAPA Rice and Beans Center in Brazil and CIRAD (formerly IRAT)

In 1992 at the beginning of the CIAT/CIRAD rice collaborative project two basic germplasm upland lines CNA IRAT 5 and CNA IRAT A were introduced in Colombia They were characterized and CNA IRAT A showed better adaptation to the Colombian upland savannas ecosystem

Site specific populations were then created by crossing CNA IRAT A with upland lines that were developed at CIAT for the savanna ecosystem This resulted in the creation of the PCT 4 population Since then PCT 4 was then used as basic germplasm to develop the population PCT 11 At the same time the introduced populations were enhanced for tolerance to blast and *Tagosodes orizicolus* In the last few years population improvement by recurrent selection has concentrated on PCT 4 and PCT 11 as well as the development of segregating lines derived from these populations

Recurrent Selection Strategy

Population breeding by recurrent selection is very efficient for improvement of traits that have low heritability Through short selection recombination cycles linkage blocks are broken down and favorable genes are accumulated This is a process of continuous improvement Numerous examples of the efficiency of the method are reported in the literature for open pollination crops like maize

Carrying a recessive male sterile gene rice populations behave partly as an open pollination crop At flowering the pollen produced by fertile plants not only pollinates itself (selfing) but

also fertilizes the flowering neighbors male sterile plants showing same flowering time plants (cross pollinating) At each cycle of population improvement fertile plants were selected for the next cycle of recurrent selection or developing segregating lines

Sowing Rice Populations

Rice populations segregate for numerous traits and were made of fertile (Msms) as well as male sterile plants (msms) allowing natural cross pollination Planting was made using individual hill plots which facilitated the identification of male sterile plants where recombination occurs To allow complete recombination between early and late flowering material two to three sowing dates were made in the same physical plot To avoid pollen contamination from other rice plots each population surrounded by a barrier of maize

Recombining and Multiplying Populations

Grains produced by male sterile plants are Ms ms and ms ms (pollen produced by fertile plants are 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

Selecting Fertile Plant for Line Development

The selection of S_0 fertile plants (Msms) was the starting point for segregating line development Each selected fertile plant was individually harvested and its seeds sown during the next season (S_1 generation) Seeds harvested on fertile plants were Ms ms Ms Ms and ms ms and the S_1 generation showed segregation of fertile (Ms ms and Ms Ms) or male sterile (msms) plants in the proportion ¾ and ¼ respectively

Through out the selection process only fertile plants were selected and harvested only fertile plants in order to eliminate the male sterile gene Advanced progenies were 100% fertile (Ms Ms) Line development follows traditional evaluation and pedigree selection

The major characteristics bred for savanna conditions were early vigor tolerance of soil acidity resistance to rice blast (*Pyricularia grisea* Sacc) and sogata rice planthopper (*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 trials in Colombia at La Libertad and Matazul Experimental Stations (LES and MES respectively)

The fields were fertilized using 300kg/ha of dolomite lime that was applied 30 days before sowing 178 kg/ha of nitrogen (59 kg/ha at 20 35 and 45 days after sowing respectively) 155 kg/ha of phosphorus at sowing and 116 kg/ha of Potassium (58 kg/ha at sowing and 29 kg/ha at 20 and 35 days after sowing respectively) No chemical control was used for pests or diseases The experimental design was of complete random blocks with 3 replications and individual plots of 5 2 m^2 (4 row of 5 m with spacing of 0 26 m) During the experiment the agronomic characteristics were recorded and the individual plots of 4 16 m^2 were harvested The yield was measured and grain quality was evaluated

Improving Rice 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 (i) mass recurrent selection and (ii) S_2 progenies evaluation For the last method fertile plants were selected and harvested in the population(s) during the cropping season 2000A and S_1 generation advanced during off season at Palmira Experimental Station (PES) After harvesting the S_2 populations they were sown at LES in 2001A and evaluated including a comparison to local checks using the augmented block design proposed by Federer Each individual S_2 progeny was made of 2 rows that were 5 m in length

Results and Discussion

The results reported here were the October 2000 March 2001 cycle at PES and during the upland rice cropping season April September 2001 at PES and MES

Line Development from Recurrent Populations

During the enhancement of populations through recurrent selection fertile plants were selected These genotypes were the starting point for the development of promising fixed lines for variety release and/or potential parents for our regional partners (Argentina Bolivia, Brazil Colombia Cuba Venezuela and the Caribbean through CRID Net)

During the cropping season at LES a total of 291 segregating and advanced lines were evaluated and selected They came from different populations in different stages of enhancement (table 1)

Table 1 List of Segregating and Fixed Lines Evaluated and Selected during the cropping Season 2001A

Population	Generation				
	S_1	S_3	S_4	S_6	S_8
PCT 4\SA\4\1	21 (15)				
PCT 4\0\0\2					
PCT 4\SA\2\1		114 (23)			
PCT 4\SA\4\1					
PCT 4 \0\0\0			44 (7)		
PCT 11\0\0\1				2 (2)	
PCT 4\SA\1\1					
PCT 4\0\0\1					
PCT A\0\0\0					110 (178)
PCT 4\0\0\1\S_3					
PCT4\PHB\1\1 PHB\1					

Line Selection by LAC NARS Breeders

The CIAT/CIRAD project jointly with EMBRAPA Brazil organized the First International Upland Rice Workshop in Villavicencio Meta Colombia in August 2000 This workshop included breeders from six countries (IP 4 Rice Annual Report 2000) and they each selected between 8 and 21% of the total number of lines (table 3)
Colombia, Brazil and Bolivia selected he most number of lines The main characteristics of the selected lines were earliness modern plant type long slender grains (of special importance for Brazil) tolerance to rice blast and potential promising yield

Table 2 Results of the Selection of Upland Lines LES Villavicencio-Meta, Colombia August 7 11 2000

Generation	Lines	Number of Selected Lines and %					
		Bolivia	Brazil	Colombia	Cuba	Venezuela	Argentina*
S_1	229	14 6 %	10 4 4%	14 6 1%	14 6 1%	5 2 2%	14 6 1%
Average 11 8 lines		Selection Intensity 5 1%					
S_2	237	0	8 3 4%	15 6 3%	0	14 5 9%	0
Average 6 2 lines		Selection Intensity 2 6%					
S_4	7	0	1 14 3%	3 43%	0	2 28 6%	0
Average 1 line		Selection Intensity 14%					
S_6	289	61 21 1%	52 18%	133 46%	47 16 2%	33 11 4%	61 21 1%
Average 64 5 lines		Selection Intensity 22%					
S_7	78	4 5 1%	20 25 6%	15 19 2%	3 3 4%	8 10 3%	4 5 1%
Average 9 lines		(11 5%)					
S_9	307	41 13 3%	66 21 5%	56 18 2%	38 12 3%	30 9 8%	41 13 3%
Average 45 3 lines		Selection Intensity 14 8%					
Total 1147		120 10 5%	157 13 7%	236 20 6%	102 8 8%	92 8%	120 10 5%
Average 137 8 lines		Selection Intensity 12%					

* Lines selected by Argentina are the same as those selected by Bolivia Bolivians breeders trained the Argentinean who was starting working with upland rice

The selected lines were harvested and the seeds shipped to each respective country s breeders A set of 147 lines was also prepared and shipped to Bolivia to be grown as core nursery demonstration plot for next years upland rice breeders workshop that will be held in Santa Cruz de la Sierra These lines show different phenotypes including plant height tillering ability cycle duration grain shape and yield potential They are representative of the variability found in segregating progenies coming from this rice population

Yield Trials

Advanced generations are promising fixed lines that passed through all agronomic selection process Selection of the best yielding lines that also showed excellent grain quality was made at LES and PES during 1999A and B semesters respectively During the cropping season 2000A a

yield trial was made at LES and it was repeated during 2001A at LES and MES (Colombian savannas)

Cropping Season 2000A at LES

Twenty four (S_4 generation) advanced lines from the first cycle of enhancement of population PCT 4 were compared to 3 checks coming from conventional breeding (Oryzica Sabana 6 released in 1992 Oryzica Sabana 10 released in 1994 and Linea 30 CIRAD 409 not yet released) The PCT 4\SA\1\1 nomenclature means one selection for Suelos Acidos Acid Soils followed by one recombination)

The grain yields ranked between 1240 and 3644 kg/ha Twenty one of the advanced lines yielded more than Oryzica Sabana 10 three more than Oryzica Sabana 6 and one more than Linea 30 (table 3) at the 0 05 level of significance There 13 lines that had yields of more than 3 T/ha as compared with Linea 30 were with 2 3 T/ha Oryzica Sabana 6 with 2 1 T/ha and Oryzica Sabana 10 with 1 2 T/ha The line PCT 4\SA\1\1>975 M 2 M 3 yielded 56% 70% and 194% more than Linea 30 Oryzica Sabana 6 and Oryzica Sabana 10 respectively The other top yield lines including PCT 4\SA\1\1>1044 M 3 M 4 and PCT 4\SA\1\1>975 M 3 M 3 while not statistically different from Linea 30 outperformed by 45% 57% and 171% Linea 30 Oryzica Sabana 6 and Oryzica Sabana 10 respectively Eleven of these high yielding lines flowered 10 to 18 days earlier than the medium cycle checks Oryzica Sabana 6 and Oryzica Sabana 10 This means that it is possible to separate earliness with from poor yield potential

To confirm the results from last year the similar yield trials were repeated in two different sites If these results confirm the observation of the 2000 trial some of these advanced lines will be tested for their utility as varieties

Table 3 Yield Trial 2000A LES Villavicencio-Meta Colombia

Line from population * PCT 4\SA\1\1	Yield (Kg/ha)	Ranking	Days to Flowering
PCT 4\SA\1\1 >975 M 2 M 3	3644	A	71
PCT 4\SA\1\1 >1044 M 3 M 4	3379	AB	74
PCT 4\SA\1\1 >975 M 3 M 3	3367	AB	74
PCT 4\SA\1\1 >975 M 3 M 4	3321	ABC	76
PCT 4\SA\1\1 >982 M 3 M 5	3277	ABC	69
PCT 4\SA\1\1 >975 M 2 M 2	3275	ABC	74
PCT 4\SA\1\1 >1479 M 1 M 6	3265	ABC	71
PCT 4\SA\1\1 >1479 M 1 M 1	3239	ABCD	78
PCT 4\SA\1\1 >975 M 3 M 2	3115	ABCD	71
PCT 4\SA\1\1 >516 M 6 M 3	3095	ABCD	80
PCT 4\SA\1\1 >1479 M 1 M 3	3028	ABCD	72
PCT 4\SA\1\1 >1479 M 1 M 5	3016	ABCD	74
PCT 4\SA\1\1 >1044 M 3 M 2	3003	ABCD	73
PCT 4\SA\1\1 >975 M 2 M 1	2947	ABCDE	71
PCT 4\SA\1\1 >1036 M 6 M 2	2868	ABCDE	71
PCT 4\SA\1\1 >1479 M 1 M 2	2832	ABCDE	67
PCT 4\SA\1\1 >540 M 3 M 5	2781	ABCDE	69
PCT 4\SA\1\1 >982 M 3 M 4	2771	ABCDE	71
PCT 4\SA\1\1 >1837 M 2 M 3	2352	BCDE	75
Linea 30 – CIRAD409	2332	BCDE	71
PCT 4\SA\1\1 >1260 M 6 M 6	2313	BCDE	75
PCT 4\SA\1\1 >1837 M 2 M 2	2273	BCDE	79
PCT 4\SA\1\1 >540 M 3 M 3	2243	CDEF	81
PCT 4\SA\1\1 >1576 M 4 M 1	2237	CDEF	79
Oryzica Sabana 6	2140	CDEF	83
PCT 4\SA\1\1 >540 M 3 M 4	1878	EF	81
Oryzica Sabana 10	1240	F	89

* PCT 4\SA\1\1 nomenclature means one selection for Suelos Acidos (Acid soils) followed by one recombination corresponding to one cycle of recurrent selection

Population Improvement

Population enhancement was made using both recurrent selection methods Mass recurrent selection on both sexes was used to select for resistance to total rice leaf blast and rice hoja blanca virus The S_2 lines were evaluated for major agronomic traits

Mass Recurrent Selection on Both Sexes for Rice Hoja Blanca Virus Rice Blast, and Major Agronomic Traits

Populations PCT 4 PCT A and PCT 5 were submitted to 3 cycles of mass recurrent selection At vegetative stage plants showing susceptibility to leaf blast and rice hoja blanca virus were eliminated without waiting until flowering to determine if they were male sterile

At harvesting time male sterile plants showing good agronomic traits were selected Seeds produced by these healthy male sterile plants were the result of fertilization by pollen produced by neighbor healthy fertile plants

Resistance to Rice Blast

The original populations of PCT 4 PCT A and PCT 5 had 43% 35% and 48% respective of susceptible plants (table 4) Starting with the first cycle of selection there was a drastic reduction in the percentage of infected plants By the third cycle of select no population had more the 0 3% infection rate with rice blast This demonstrates the ability of this recurrent selection method to rapidly increase the level of resistance to this disease

Table 4 Total Leaf Blast Resistance of the Enhancement Populations PCT 5, PCT A and PCT-4 PES 1999B

Cycles of Mass Recurrent Selection	Year of Evaluation	PCT 5	PCT A	PCT-4
Basic Population	1995	47 8 *	35 3	42 7
First cycle	1996	1 5	1 0	0 5
Second cycle	1997	3 7	3 3	4 5
Third cycle	1998	0 3	0 2	0 1

* % of plants with leaf blast symptoms

Resistance to Rice Hoja Blanca Virus

After 3 cycles of mass recurrent selection 107 S_2 lines from the 3 populations were evaluated in 1999 at PES for resistance to RHBV The result of the S_2 evaluation of the enhanced populations showed that 54% have resistance to RHBV (table 5) Another 43% were rated as intermediate for resistance to RHBV

Table 5 Resistance to Rice Hoja Blanca Virus of the Enhancement Populations PCT 5, PCT A and PCT-4

	Reaction to Rice Hoja Blanca Virus (1 9 scale)		
	Resistant (1 3)	**Intermediate (5)**	**Susceptible (7 9)**
S_2 lines of enhanced populations	54 2*	42 9	2 8
FEDEARROZ Lines	59 1	30 6	10 2
ICA Lines	51 4	4 0	44 4
IRRI Lines	5 6	4 6	89 7
Colombia 1 (R check)**	90 3	9 7	0 0
Bluebonnet (S check)**	0 0	3 8	96 2
CICA 8 (I check)**	0 0	86 4	13 6

* % of evaluated lines
** R S and I Resistant Susceptible and Intermediate

Recurrent Selection Based on S_2 Line Evaluation

The enhanced populations are considered good reservoirs of genotypes for the development of resistant fixed lines During the cropping season 2001A the 3 enhanced populations were cropped at LES and S_0 fertile plants selected for line development

Population PCT-4\SA\3\1

After the first selection cycle for acid soil tolerance (SA) population PCT 4 was recombined 3 times (\3\) The resulting population was grown at LES during 2000 A and S_0 fertile plants were selected The generation S_1 was grown and evaluated at LES during the cropping season 2001A

Population PCT-4\SA\1\1, SA\1

Population PCT 4\SA\1\1 with one cycle of recurrence (SA\1) was submitted to a second cycle of recurrent selection The resulting enhanced population (PCT-4\SA\1\1 SA\1) was grown during the year 2000 at LES A third cycle of recurrent selection was started through the selection of S_0 fertile plants Generation S_1 was grown at PES during off season 2000B and the S_2 evaluated during 2001 A at LES

Genetic Progress Evaluation through Recurrent Selection Process Study Case of Population PCT-4

As part of the Master Degree thesis of Yolima Ospina the genetic progress for acid soil tolerance and main agronomic traits (flowering time plant height and grain yield components) after one selection cycle using different recombination cycles were evaluated

Materials and Methods

The starting source for this experiment was the population PCT 4 The S_1 lines were grown in two plots with contrasting soil acidity The experimental design was augmented Federer blocks composed by S_1 lines from 4 populations and the 3 susceptible checks of CICA 8 CICA 9 and Oryzica Llanos 5 as well as the 3 tolerant checks of Oryzica Sabana 6 Oryzica Sabana 10 and CIRAD 409 to soil acidity The data has been collected and is currently being evaluated

Registering New Site-Specific Upland Composite Populations

Since 1993 and following a recommendation of the First International Upland Rice Breeders Workshop held in Montpellier–France the CIAT/CIRAD Rice Collaborative Project is in charge of managing a registration catalogue were rice populations are described including genetic constitution and process of creation Each rice breeder involved in population breeding can apply for registering the population he develops The catalogue is annually issued and circulated

In 2001 new site–specific populations were registered on request from two breeders Dr Tao Dayun from FCRI/YAAS Province of Yunnan China who applied for the registration of a Japonica population showing restoring ability and good grain quality The germplasm was registered as PYN 3 and was developed from the CIAT/CIRAD population PCT 5 Dr Michel Vales from CIAT/CIRAD Rice Project Cali Colombia who applied for the registration of recurrent populations with narrow genetic base

Upland Line Registration

CIRAD has a mechanism by which breeders can register specific material not necessarily release as variety It receives a consecutive CIRAD catalogue number and the corresponding local identification where it was bred In 2001 we applied for the registration of the upland savanna line PCT 4\SA\1\1 >975 M 2 M 3 that performed very well in the 2000 LES yield trial

Upland Population Breeding by LAC NARS

Population breeding activities directly conducted by NARS and monitored by the CIAT/CIRAD project can be found in the book titled Advances in Rice Population Breeding (Avances en el Mejoramiento Poblacional en Arroz) published in 2000 by CIAT/CIRAD (Colombia) EMBRAPA (Brazil) and DANAC Foundation (Venezuela) Scientists from Brazil Bolivia Colombia China Cuba and El Salvador reported on their upland rice breeding research activities

Conclusions

Populations with broad genetic base were developed and enhanced By using mass recurrent selection 3 populations were enhanced for leaf blast and RHBV resistance They are good reservoirs of potentially good genotypes for line development Selection of S_0 fertile plants was made Enhancement of population PCT 4 is continues using S_2 line evaluation selection and recombination

During population enhancement fertile plants were selected and their progenies evaluated and selected by traditional pedigree method Three very promising lines were identified They showed high yielding potential and were as early as Linea 30 CIRAD 409 and 10 to 18 days earlier than the medium cycle checks Oryzica Sabana 6 and Oryzica Sabana 10

Many of the lines from the recurrent selection populations yielded 40 70% more than Linea 30 and the best local check Oryzica Sabana 6 These results demonstrate that it is possible to break down the correlation between earliness and low yield potential

Future activities

These enhanced upland population will continue to be evaluated for agronomic and pest resistance traits These lines will be extracted evaluated and selected

There are many promising upland lines that will be tested in multilocation trials to confirming their behavior in farm conditions This will be done in conjunction with CORPOICA and should be the basis for new varieties

- **Lowland Rice Ecosystems**

M Chatel and Y Ospina

Abstract

As for upland rice CIAT and CIAT/CIRAD rice project concentrates on broadening the genetic base of lowland rice The development of lowland rice populations with broad genetic base and their enhancement through recurrent selection were new breeding strategies to achieve this objective

Using a recessive male sterile gene (ms) from a mutant of IR36 the development of rice population was eased 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 (Southern Cone of Latin America) were developed with NARS They are the starting point of rice recurrent selection projects Networking activities are made through GRUMEGA Network and FLAR

Key words Lowland rice rice populations genetic base male sterile gene enhancement site specific populations and recurrent selection

Introduction

Population Breeding for Lowland irrigated rice is made in close collaboration with FLAR partners in Latin America (CIRAD is a member of FLAR) and CIRAD partners in Europe and Asia

The breeding population project started by introducing to Colombia different gene pools and populations previously developed in Brazil by CIRAD/EMBRAPA –Rice and Beans Center and by CIRAD in French Guyana

Germplasm was characterized at Palmira Experimental Station Valle Colombia (PES) The best adapted ones were used to develop new populations This resulted in three *indica* populations that were registered in the recurrent selection catalogue as PCT 6 PCT 7 and PCT 8 This work was conducted at CIAT with Drs C Martinez and E P Guimarães

A *japonica* population developed by CIRAD for temperate areas was registered as GPIRAT 10 From late 1996 this basic germplasm was dispatched to our regional partners and outside Latin America It was the starting point of population breeding activities in different countries

In 1999 the II International Workshop on Rice Recurrent Selection held in Goiania Brazil was the occasion for the regional NARS to present updated information on the use of population breeding A book edited by Dr Elcio Guimarães was published jointly by CIRAD/CIAT (Colombia) EMBRAPA (Brazil) and the DANAC Foundation (Venezuela)

At the Workshop final plenary session it was decided to create a formal group named Rice Advanced Rice Genetic Breeding Group (Grupo de Mejoramiento Genetico Avanzado de Arroz – GRUMEGA) coordinated by CIAT/CIRAD and EMBRAPA Rice and Beans Center

Activities presented here after were conducted at PES and correspond to special services provided to NARS Population breeding activities directly conducted by NARS and monitored by the CIAT/CIRAD project can be found in the book untitled Advances in Rice Population Breeding (Avances en el Mejoramiento Poblacional en Arroz) published in 2000 by CIAT/CIRAD (Colombia) EMBRAPA (Brazil) and DANAC Foundation (Venezuela)

Argentina, Brazil Chile China, Colombia Cuba, Venezuela and Uruguay are reporting research activities on lowland rice population breeding

Materials and Method

In 1996 populations crated by CIAT/CIRAD were shipped to regional NARS for evaluation and selection From the best adapted germplasm new site specific populations were developed by introduction of local variability The site specific populations were registered in the recurrent selection catalogue CIAT/CIRAD populations were seed increased at PES to ensure good disposability of fresh seed

Results and Discussion

Developing Site-Specific Composite Populations

Argentina

During 2000 and the first semester of 2001 a new population was developed at CIAT by introducing into the population PCT 8 six parents selected by the Argentineans
The new site specific population was identified as PARG 3 and shipped to Argentina where it will be grown during the cropping season 2001/2002

Chile

A new population is being developed in Chile by INIA It is targeting cold tolerance grain quality and yield potential It is identified as PQUI 2

Uruguay

The Uruguayan breeder dealing with population breeding is currently in the US and his return to Uruguay is planned for next year Before he leaves Uruguay it was decided to create 3 site specific populations with the following objectives
One is for short/medium grain breeding (export market to Asia)
One is for long slender grain and high quality (export market to Middle East)
One is for long term enhancement
The 3 populations were developed at CIAT Palmira and were identified as PURG 1 PURG 2 and PURG 3 They are ready for shipping

China

The Food Crops Research Institute (FCRI) of the Yunnan Academy of Agricultural Sciences (YAAS) has developed a site specific *japonica* population both for lowland and upland conditions The male sterile source is from the CIAT/CIRAD upland population PCT 5 crossed with 8 lines (2 from China with restoring gene Rf 1 3 inter specific lines from WARDA and 3 CIRAD upland lines)
The new site specific population is identified as PYN 3

France and Chile

In Europe there is a market for scented rice and the consumers are will to pay extra for this rice which makes this a crop of high added value for rice producers Rice aroma is a very difficult trait to breed by conventional crossbreeding It is polygenic and involves major and minor genes Population breeding is a suitable method to enhance the characteristic The development of a new population for temperate areas started during the second semester of 2001

The new germplasm correspond to a new population by introduction into the Chilean population PQUI 1 (well adapted to temperate climate) of 26 aromatic lines selected by CIRAD France At PES individual crosses were made between each aromatic line and male sterile plants of the Chilean population

The new population is to be characterized and enhanced both in France (Camargue rice growing region) and Chile Exchange of information and breeding material will be made between both breeding projects

Colombia

Two new composite populations with inter specific lines developed by Dr Cesar Martinez from CIAT are under way Inter specific selected lines were crossed with PCT 6 and PARG 3 for tropical and sub tropical conditions In relation to existing composite populations they were characterized by new original genetic variability Enhancement will at first be made in Colombia

Maintaining Composite Populations

Because we manage the catalogue for rice germplasm for recurrent selection we also have the responsibility to ensure presence of sufficient seed in the germplasm bank During the second semester of 2001 at PES we started the multiplication of different populations

Line Development from PCT 6

Population PCT 6 was enhanced by Dr Michel Valès (CIAT/CIRAD Project) both for total and partial resistance to rice blast and main agronomic traits The first cycle of enhancement is considered as a reservoir for fixed line development During 2001A the enhanced population was grown at Santa Rosa Experimental Station under blast pressure and 148 sterile plants were selected for recombination

Registering new Populations

In 2001 we registered the site specific populations from NARS
Argentina PARG 3
Uruguay PURG 1 PURG 2 and PURG 3
China PYN 3
CIAT/CIRAD (M Vales)

Conclusions

Regional LAC NARS have a strong programs in population breeding We are collaborating with their efforts to developing site specific populations and rice enhancement projects

Future Activities

The development of new site specific population is an on going activity The monitoring and networking of population breeding activities for site specific populations will continue to assure their development into advanced lines with the range of characteristic needed at each local

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OUTPUT 1 ENHANCING GENE POOLS

1B Developing Upland Rice for Small Landholders

- **New Recurrent Populations**

M Vales J Garciaa, J Dossmann

Abstract

Crosses to form new recurrent populations with broad genetic base are in way to respond to partner new demands

- Haiti (MARNDR) upland rice population for hillsides including resistance to rice blast disease and to the mite/fungus complex *Steneotarsonemus spinki/Sarocladium oryzae*
- Cuba (IIA) Lowland rice population including resistance to rice blast disease cold salinity *Rhizoctonia solani* and the mite/fungus complex *Steneotarsonemus spinki/Sarocladium oryzae*
- Colombia (FEDEARROZ) lowland population including resistance to *Rhizoctonia solani* and some cold tolerance
- CIAT CIRAD collaborative project (C Martinez and M H Chatel) lowland population including interspecific progenies

The use of recurrent population with narrow genetic base is a success in Venezuela (DANAC) Colombia (FEDEARROZ) and Brazil (CIRAD/Agronorte) So new populations are in way to be formed to respond to new partner demands

Haiti (MARNDR) inundated rice population for costal area including resistance to rice blast disease salt tolerance and to the mite/fungus complex *Steneotarsonemus spinki/Sarocladium oryzae*

UMATA of Guapi and Timbiqui population including tolerance to low sun light and salinity for the strands of the Colombian costal streams

New Recurrent Population with Broad Genetic Base

Because it is the adapted method to improve polygenic traits recurrent selection has been proposed as a breed method for rice since 1982 (Vales 1983 and 1987) Recurrent populations with broad genetic base permit greater diversity in breeding programs To respond to partner new demands recurrent selected populations are being developed for upland rice for hillsides in Haiti (MARNDR) and the some of the important traits include resistance to rice blast disease and to the mite/fungus complex *Steneotarsonemus spinki/Sarocladium oryzae* (Table 1) For Cuba (IIA)

a lowland rice population which carries resistance to rice blast disease cold salinity *Rhizoctonia solani* and the mite/fungus complex *S spinki/S oryzae* (Table 2) For FEDEARROZ in Colombia a lowland population that carries resistance to *Rhizoctonia solani* and cold tolerance (Table 3) is being developed CIAT CIRAD collaborative project (C Martinez and M H Chatel) has a lowland population that includes interspecific progenies (Table 4) with the Argentinean population PARG 3\0\0\1

Table 1 Progenitors to Form an Upland Rice Population for Haitian Hillsides

Variety	Seed Origin	Interest
ARC 7117	IRGC 20516	R [1] *Steneotarsonemus spinki*
CHIANUNG SEN 8	IRGC 26954	R *Steneotarsonemus spinki*
CHIANUNG SEN 11	IRGC 26955	R *Steneotarsonemus spinki*
CHIANUNG SENYU 19	CIRAD France	R *Steneotarsonemus spinki*
ER BAI AI	IRGC 67110	R *Steneotarsonemus spinki*
INTAN	CIRAD France	R *Steneotarsonemus spinki*
INTAN	IRGC 4230	R *Steneotarsonemus spinki*
RAM TULASI	CIRAD France	R *Steneotarsonemus spinki*
RAM TULASI	IRGC 181	R *Steneotarsonemus spinki*
RAM TULSI	IRGC 10058	R *Steneotarsonemus spinki*
TAICHUNG 189	IRGC 72922	R *Steneotarsonemus spinki*
TAICHUNG SEN 5	IRGC 38893	R *Steneotarsonemus spinki*
TAINAN 6	IRGC 2957	R *Steneotarsonemus spinki*
TAINAN 6	IRGC 28629	R *Steneotarsonemus spinki*
TAINUNG 67	IRGC 47743	R *Steneotarsonemus spinki*
TP SEL (B 52 4820)18	IRGC 5699	R *Steneotarsonemus spinki*
LATSIBAVY	CIRAD FOFIFA Madagascar	R *Sarocladium oryzae*
LATSIDAHY	CIRAD FOFIFA Madagascar	R *Sarocladium oryzae*
CIRAD 391	CIRAD FOFIFA Madagascar	R *Sarocladium oryzae*
CIRAD 392	CIRAD FOFIFA Madagascar	R *Sarocladium oryzae*
CIRAD 407	CIRAD FOFIFA Madagascar	R *Sarocladium oryzae*
CIRAD 408	CIRAD FOFIFA Madagascar	R *Sarocladium oryzae*
IRAT 13	CIRAD France	R *Pyricularia oryzae*
IRAT 104	CIRAD France	R *Pyricularia oryzae*
MOROBEREKAN	CIRAD France	R *Pyricularia oryzae*
ORYZICA LLANO 5	CIAT	R *Pyricularia oryzae*
CIRAD 403	CIRAD France	R drought
CIRAD 409	CIRAD CIAT	R acid soil early
CIRAD 400	CIRAD France	Grain quality
MERVEILLEUX	CIRAD France	Grain quality

1 R = Resistance to

Table 2 Progenitors to Form an Upland Rice Population for Haitian Hillsides

Variety	Seed Origin	Interest
ARC 7117	IRGC 20516	R [1] *Steneotarsonemus spinki*
CHIANUNG SEN 8	IRGC 26954	R *Steneotarsonemus spinki*
CHIANUNG SEN 11	IRGC 26955	R *Steneotarsonemus spinki*
CHIANUNG SENYU 19	CIRAD France	R *Steneotarsonemus spinki*
ER BAI AI	IRGC 67110	R *Steneotarsonemus spinki*
INTAN	CIRAD France	R *Steneotarsonemus spinki*
INTAN	IRGC 4230	R *Steneotarsonemus spinki*
RAM TULASI	CIRAD France	R *Steneotarsonemus spinki*
RAM TULASI	IRGC 181	R *Steneotarsonemus spinki*
RAM TULSI	IRGC 10058	R *Steneotarsonemus spinki*
TAICHUNG 189	IRGC 72922	R *Steneotarsonemus spinki*
TAICHUNG SEN 5	IRGC 38893	R *Steneotarsonemus spinki*
TAINAN 6	IRGC 2957	R *Steneotarsonemus spinki*
TAINAN 6	IRGC 28629	R *Steneotarsonemus spinki*
TAINUNG 67	IRGC 47743	R *Steneotarsonemus spinki*
TP SEL (B 52-4820)18	IRGC 5699	R *Steneotarsonemus spinki*
LATSIBAVY	CIRAD FOFIFA Madagascar	R *Sarocladium* sp R cold
LATSIDAHY	CIRAD FOFIFA Madagascar	R *Sarocladium* sp R cold
CIRAD 391	CIRAD FOFIFA Madagascar	R *Sarocladium* sp R cold
CIRAD 392	CIRAD FOFIFA Madagascar	R *Sarocladium* sp R cold
CIRAD 407	CIRAD FOFIFA Madagascar	R *Sarocladium* sp R cold
CIRAD 408	CIRAD FOFIFA Madagascar	R *Sarocladium* sp R cold
IRAT 13	CIRAD France	R *Pyricularia oryzae*
IRAT 104	CIRAD France	R *Pyricularia oryzae*
MOROBEREKAN	CIRAD France	R *Pyricularia oryzae*
ORYZICA LLANO 5	CIAT	R *Pyricularia oryzae*
PALMAR	DANAC	R *Rhizoctonia solani*
FONAIAP 1	DANAC	R *Rhizoctonia solani*
REMADJA	CIAT	R *Rhizoctonia solani*
PANKAI	CIAT	R *Rhizoctonia solani*
TADUKAN	CIAT	R *Rhizoctonia solani*
TA PO CHOO Z	CIAT	R *Rhizoctonia solani*
TETEP	CIAT	R *Rhizoctonia solani*
REMADJA	CIAT	R *Rhizoctonia solani*
CIRAD 400	CIRAD France	Grain quality
MERVEILLEUX	CIRAD France	Grain quality

1 R = Resistance to

Table 3 Progenitors Used to Form an Lowland Population for Colombia

Variety	Seed Origin	Interest
LATSIBAVY	CIRAD FOFIFA Madagascar	Cold tolerance
LATSIDAHY	CIRAD FOFIFA Madagascar	Cold tolerance
CIRAD 391	CIRAD FOFIFA Madagascar	Cold tolerance
CIRAD 392	CIRAD FOFIFA Madagascar	Cold tolerance
CIRAD 407	CIRAD FOFIFA Madagascar	Cold tolerance
CIRAD 408	CIRAD FOFIFA Madagascar	Cold tolerance
IRAT 13	CIRAD France	R [1] *Pyricularia oryzae*
IRAT 104	CIRAD France	R *Pyricularia oryzae*
MOROBEREKAN	CIRAD France	R *Pyricularia oryzae*
ORYZICA LLANO 5	CIAT	R *Pyricularia oryzae*
PALMAR	DANAC	R *Rhizoctonia solani*
FONAIAP 1	DANAC	R *Rhizoctonia solani*
REMADJA	CIAT	R *Rhizoctonia solani*
PANKAI	CIAT	R *Rhizoctonia solani*
TADUKAN	CIAT	R *Rhizoctonia solani*
TA PO CHOO Z	CIAT	R *Rhizoctonia solani*
TETEP	CIAT	R *Rhizoctonia solani*
CIRAD 400	CIRAD France	Grain quality
MERVEILLEUX	CIRAD France	Grain quality
FEDEARROZ 50	FEDEARROZ	R Hoja blanca and Sogata
FEDEARROZ 2000	FEDEARROZ	Yield

1 R = Resistance to

Table 4 Interspecific Progenies Proposed by C Martínez

Pedigree	Progenitors
CT13941 11 M 25 5 M M	*O rufipogon* / *2 Bg90 2
CT13946 26 M 5 3 M M	*O rufipogon* / *2 Bg90 2
CT13958 13 M 26 4 M M	*O rufipogon* / *2 Bg90 2
CT13956 29 M 29 2 M M	*O rufipogon* / *2 Bg90 2
CT13959 3 M 10 4 M M	*O rufipogon* / *2 Bg90 2
CT13976 7 M 14 1 M M	*O rufipogon* / *2 Bg90 2
CT14938 36 1 M 1	*O barthii* / 3* Lemont

New Recurrent Population with Narrow Genetic Base

Recurrent populations with narrow genetic base was proposed for obtaining results more quickly than is possible with a population with broad genetic base (Vales 1998) Some narrow based recurrent populations are being used by our partners

Brazil	PCT 18 for upland rice (James Taillebois CIRAD Agronorte)
Venezuela	PCT 16 for tropical lowland rice (Carlos Gamboa DANAC)
Colombia	PCT 15 for favorable upland rice and PCT 16 (Edgar Corredor FEDEARROZ)
	PCT 14 for altitude lowland rice and PCT 17 for hillsides (Own program for Confronting food insecurity in the hillsides see Output 3)

A narrow genetic basis recurrent selected population is being made for an inundated rice population for the Haitian coastal areas which includes resistance to rice blast disease salt tolerance and to the mite/fungus complex *Steneotarsonemus spinki/Sarocladium oryzae* (Table 5) Another population in being made for the strands of the Colombian coastal streams of Guapi and Timbiqui which includes tolerance to low sun light and salinity (Table 6)

Table 5 Progenitors to Form an Inundated Rice Population for Haitian Coast

Variety	Seed origin	Interest
RAM TULASI	CIRAD France	R *Steneotarsonemus spinki*
IRAT 13	CIRAD France	R *Pyricularia oryzae*
IRAT 112	CIRAD France	R drought
LATSIDAHY	CIRAD FOFIFA Madagascar	R *Sarocladium oryzae*
MALAYIKA	MARNDR Haiti	Tolerance to *Paille Noire*
CAMPECHE	MARNDR Haiti	Salinity tolerance
MADAME TISON	MARNDR Haiti	Adaptation to acid soil
LA CRETE A PIERROT	MARNDR, Haiti	Taste preference

R Resistance to

Table 6 Progenitors to Form an Inundated Rice Population for Colombian Coast

Variety	Seed Origin	Interest
IRAT 216	CIRAD France	Upland/lowland adaptability
CHINO GRANDE	UMATA Guapi Colombia	Low sun light tolerance
FEDEARROZ 2000	FEDEARROZ Colombia	Yield
CAMPECHE	MARNDR Haiti	Salinity tolerance

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OUTPUT 1 ENHANCING GENE POOLS

Improving rice germplasm for Latin America and the Caribbean (LAC) by the IP 4 Project at CIAT is being accomplished through the increase of genetic diversity and enhancement of gene pools for higher more stable yields with adequate levels of resistance to pathogen and insect pests relevant to the region CIAT breeding strategies focus on developing and improving populations to provide our partners with sources of potential parents having specific traits or populations from which they may select and advance fixed lines for release as commercial varieties

In the case of irrigated and favored upland ecosystems this is accomplished through the characterization and utilization of wild rice species development of improved populations through recurrent selection methods and the introgression of agronomic traits from the IRRI new plant type into our local gene pool

1 C Broadening the Genetic Base of Irrigated Rice in Latin America

- **Utilization of Wild Rice Species for Enhancing Gene Pools**

Introduction

Oryza wild species represent a potential source of new alleles for improving yield quality and stress resistance of cultivated rice Still effective use of wild species genes remains largely unexplored Advanced backcross breeding schemes (Tanskley and Nelson 1996) using molecular mapping techniques represent an alternative to reduce the genetic background from wild species parentals and to rapidly discover and transfer valuable alleles from the wild species into elite rice varieties

This collaborative project between CIAT WARDA and Cornell University is aimed at characterizing and utilizing the genes from rice wild species for the improvement of cultivated rice Two approaches are being followed molecular characterization of selected inter specific populations and the evaluation and selection of inter specific populations via conventional breeding methods

- **Molecular Characterization of Selected Inter Specific Populations**

The main objective of these activities is to carry out the molecular characterization of populations resulting from wide crosses in order to identify alleles associated with traits of agronomic importance

- **Quantitative trait loci for yield and yield components in an *Oryza sativa* x *Oryza rufipogon* BC_2F_2 population evaluated in an upland environment**

P Moncada C P Martinez J Borrero M Chatel H Gauch Jr E Guimarães J Tohme and S R McCouch

Abstract

An advanced backcross breeding strategy was used to identify quantitative trait loci (QTLs) associated with eight agronomic traits in a BC_2F_2 population de rived from an interspecific cross between Caiapo an upland *Oryza sativa* subsp *japonica* rice variety from Brazil and an accession of *Oryza rufipogon* from Malaysia This study were made to determine whether trait enhancing QTLs from *O rufipogon* would be detected in the BC_2F_2 families grown under the drought prone acid soil conditions to which Caiapo was adapted Another objective was to compare putative QTL containing regions identified in this study with those previously reported for populations adapted to irrigated lowland conditions Based on analyses of the SSLP and RFLP markers distributed throughout the genome and using single point interval and composite interval mapping two putative *O rufipogon* derived QTLs were detected for yield 13 for yield components four for maturity and six for plant height We conclude that advanced backcross QTL analysis offers a useful germplasm enhancement strategy for the genetic improvement of cultivars adapted to stress prone environments

Introduction

This work is a summary of the results that were publish this year (Moncada et al 2001) Cultivated rice *(Oryza sativa* L) is a genetically diverse species with broad adaptation to a wide range of growing environments *O rufipogon* consists of accessions that are able to prosper under conditions of both complete water saturation (anaerobic soils) and water deficit (as in upland environments) (Morishima et al 1962) and is found in both tropical and temperate growing conditions While a partial sterility barrier separates the indica and japonica subspecies both cross readily with most accessions of *O rufipogon* The possibility of selectively introgressing useful genes from *O rufipogon* to rice cultivars suggests a way of improving the performance of *O sativa* while simultaneously broadening the genetic base of cultivated rice Although upland rice constitutes about 12% of the total area planted to rice on a global basis it is the dominant form of rice culture in many parts of Latin America and most of West Africa In Latin America as a whole about 45% of rice is produced as an upland crop and two thirds of it is in Brazil s savannas where large mechanized farms predominate Caiapo was one of the most widely planted varieties in this region of Brazil covering 32% of the upland acreage in 1996 Grown mostly in low pH soils that are prone to water deficit yields of Caiapo typically average 2 5 t/ha Therefore breeding to increase the productivity of upland rice is of interest for both ecological and economic reasons

The genetic base of rice in America is narrow due to the fact that a small core of adapted progenitors has been used repeatedly in rice breeding programs in Latin America, the Caribbean and North America (Hargrove et al 1980 Dilday 1990 Cuevas Perez et al 1992 Guimaraes 1993) New resources can provide genetic variation for future advance in plant breeding

Advanced backcross (AB) QTL analysis (Tanksley and Nelson 1996) can be used to evaluate mapped donor introgressions in the genetic background of an elite recurrent parent Using this approach specific regions of the genome derived from either wild or adapted sources of germplasm and tagged with molecular markers can be associated with the performance of segregating off spring In this study we developed a population using an accession of *O rufipogon* (IRGC # 105491) as a donor with Caiapo as the recurrent parent We evaluated 274 BC_2F_2 families under low input conditions in an upland site in Colombia, with and without pasture competition We were interested in determining whether *O rufipogon* derived QTL alleles would be associated with positive or negative effects under these cultural conditions and in comparing the putative QTL containing regions with those previously found in favorable rice growing environments

Materials and Methods

Plant Materials

Caiapo was used as the recurrent parent in this study It typically yields 2 5 t/ha, is approximately 80 cm tall has good physical and edible grain quality tolerance to leaf blast *(Pyricularia oryzae*) moderate resistance to neck blast and tolerance to aluminum toxicity acidic soil conditions and drought *O rufipogon* (IRGC #105491) is a wild accession from Malaysia It is approximately 95 cm tall has resistance to rice blast small seeds with dark hulls that shatter fairly easily and heads about the same time as Caiapo under the conditions used in this study

Population development

A single plant of *O rufipogon* served as female in crosses to several Caiapo individuals The three most vigorous F_1 hybrid plants were backcrossed to Caiapo (as the female) from which 224 BC_1F_1 seeds were obtained The resulting BC_1F_1 plants were transplanted under irrigated conditions to ensure survival during population development Phenotypic selection was performed to eliminate sterility very late flowering spreading plant type excessive shattering long awns dark hull color and excessively tall plants The best 40 individuals were backcrossed to Caiapo and approximately 30 BC_2F_1 seeds per plant were produced Twenty BC_2 seeds from each of the 40 BC_1 plants were sown in wooden trays in the screen house and later transplanted under irrigated conditions to allow optimal seed production during selfing The best 300

individuals were selected based on phenotype and harvested individually to generate BC_2F_2 seed Agronomic traits were measured for all 300 BC_2F_2 families however only 274 families were used to collect molecular marker data because of non germination of seeds for 26 families

Field Trials

Two different experiments with two replications each were performed in the experimental station at Villavicencio Colombia In the first experiment 300 BC_2F_2 families were established as an upland monoculture The soil was amended with calcium carbonate before planting For fertilization 60 kg of N/ha 60 kg K_2 O/ha and 60 kg of P_2O_5 /ha were applied The weeds were controlled through applications a post emergence herbicide and by manual weeding The fungicide Bim was applied throughout the vegetative cycle as a preventive disease control measure

Trait Evaluations

Ten plants were selected at random from each of the 300 BC_2F_2 families in each experiment and evaluated for eight agronomic traits including days to heading plant height panicle length number of panicles per plant percent sterility grains per plant 1000 grain weight and yield per plant

Marker Analyses

The population of 274 BC_2F_2 families was analyzed using a total of 125 markers distributed at approximately 10 cM intervals throughout the genome A total of 200 restriction fragment length polymorphisms (RFLPs) using 4 restriction enzymes (*Eco*RI *EcoRV Hin*dIII and *Dra*I) and 50 simple single length polymorphisms (SSLPs) were used to survey the parents for polymorphism The RFLP markers used to do the parental survey were chosen based on genome distribution and previous experiments including the set of Cornell Anchor Probes widely used for comparative mapping as described by Van Deynze et al (1998) and many of the markers previously used for polymorphism analysis (Rice Genes database http //genome cornell edu/rice/) The SSLPs were chosen to close gaps in the linkage map left by the distribution of RFLPs

Linkage map

The order of the RFLP markers was based on the interspecific map of rice described by Causse et al (1994) and the order of SSLP markers was based on Chen et al (1997) and Temnykh et al

(1999) Marker integration was done by aligning markers common to both populations and establishing the most likely order and cM distances using Mapmaker on the BC_2F_2 population Segregation ratios of individual markers were statistically determined for each marker locus and deviation from the expected Mendelian ratios was determined by X^2 tests ($P < 0.01$)

QTL analysis

Nomenclature for QTLs was as described in McCouch et *al* (1997) The association between phenotype and marker genotype was investigated using single point analysis (SPA) interval map ping (IM) and composite interval mapping (CIM) For single point analysis and interval mapping the QGENE application (Nelson1997) was used Composite interval mapping (Liu 1997) was implemented using QTL Cartographer software (Basten et al 1994 1997) with a model specifying five cofactors (as recommended by the authors of the software as default) to control for genetic background and a window size of 10 cM that blocked out a region of 5 cM on either side of the markers flanking the test site

The experiment wise threshold for composite interval mapping was obtained by doing 1000 permutations at $P < 0.01$ with an average likelihood ratio of 17 5 corresponding to a LOD score of 3 80 Confidence limits were calculated for the threshold values corresponding to each individual trait for both IM and CIM (data not shown) and because the distributions overlap in both analyses we used the average threshold for all traits (Mood et al 1974) QTLs identified using these significant thresholds did not always agree for each analytical method Those reported in this study represent QTLs identified by at least two of the methods described above

Genotype by environment interactions were analyzed using a standard analysis of variance (Proc GLM SAS 1988) with $P < 0.01$ The rank of the different families in both environments were tested to see if there were shifts in population means using Spearman rank correlation coefficient (r_s) with a $P < 0.01$

Results

Trait Correlation s

The strongest correlation was found between yield and grains per plant with significant correlations also found between yield and the number of panicles per plant plant height panicle length and 1000 grain weight (Table 1) There was a negative correlation between yield and days to heading that can be explained by the fact that drought differentially affected the late maturing genotypes depressing yields while early maturing lines escaped the most serious effects of the drought

Phenotypic variation for the different traits

Positive phenotypic transgressive variation in the BC_2F_2 population was observed for all traits except for the number of panicles per plant For this trait *O rufipogon* demonstrated a significantly higher number of panicles per plant than Caiapo or any of the BC_2F_2 families in both experiments The transgressive variation suggested positive genotype x genotype (G xG) variation where *O rufipogon* alleles augment performance in a largely Caiapo genetic background

QTL Regions

Significant QTLs were detected for all traits except panicle length as summarized in Table 2 Of the 25 QTLs identified SPA and IM detected 23 (92%) QTLs in common Seventeen (68%) of the QTLs detected by SPA were also detected by CIM while 76% of QTLs detected by IM were detected by CIM The lack of consensus regarding QTL identification was most extreme for plant height where four QTLs identified by both SPA and IM were not significant using CIM This is a case where the use of cofactors appears to be particularly important However it is interesting to note that when the four plant height QTLs not detected by CIM are removed from the comparison 17 (81%) and 19 (90%) of the remaining QTLs for all traits detected by SPA and IM respectively were also detected by CIM In many of these cases a sub threshold peak (indicated by regular font in Table 2) was detected for the alternate experiment QTLs at or above the empirical threshold are in bold in Table 2

Table 1 Correlation coefficients among traits in an *O rufipogon* derived BC_2 population (*=p<0 05, **=p<0 01)

Item	Days head	Plant height	Pan Length	Pan /pl	Percent sterility	Gr/pl	1000-gw	Plant yield
Days to heading	1							
Plant height	–0 31**	1						
Panicle length	–0 08	0 50**	1					
Number pan /pl	–0 22**	0 09	0 03	1				
Percent sterility	0 31**	–0 13	–0 09	–0 13	1			
Grains/plant	–0 39**	0 47**	0 47**	0 60**	–0 43**	1		
1000 grain weight	–0 12*	0 22	0 09	0 25**	–0 04	0 23**	1	
Plant yield	–0 40**	0 48**	0 44**	0 61	–0 41**	0 97	0 43**	1

Table 2 Putative QTLs Detected in a BC_2 Population from a Caiapo/*O rufipogon* Cross

QTL	Chrom	Marker	Exp	Increased effect	F SPA	P SPA	LOD SPA	Var SPA	LOD IM	Var IM	LOD CIM	Var CIM
Days to heading												
Dth2 1	2	RM266 RM207	1	rufipogon	14 51	0 0002	3 08	0 054	3 49	0 125	4 65	0 140
Dth3 1	3	RG104 RZ329	1	rufipogon	25 01	0 0000	5 23	0 085	5 18	0 088	5 94	0 097
Dth3 2	3	RZ576 RZ22	1	Caiapo	15 37	0 0001	3 26	0 055	3 31	0 053	3 88	0 064
Dth7 1	7	RG30 RM125	1	rufipogon	21 40	0 0000	4 50	0 073	4 88	0 116	4 12	0 078
Plant height												
Ph1 1	1	RG462 RZ613	1	rufipogon	11 86	0 0007	2 54	0 042	3 74			
	1	RZ613 RZ513	1	rufipogon	5 51	0 0196	2 65	0 020	3 65	0 095		
Ph1 2	1	RM104 RZ801	1	rufipogon	16 99	0 0001	3 60	0 063	4 18	0 118	3 76	
Ph2 1	2	RG256b- RM207	1	rufipogon	8 03	0 0050	1 73	0 029	2 06		2 02	
Ph4 1	4	RG169 CDO244	1	rufipogon	11 96	0 0006	2 56	0 042	4 57			
	4	CDO244 RZ740	1	rufipogon	10 81	0 0011	2 31	0 039	2 59			
Ph5 1	5	CDO202 RZ925	1	Rufipogon	14 61	0 0002	3 11	0 052	3 01	0 059		
	5	CDO202 RZ925	1	rufipogon	37 49	0 0000	7 67	0 124	7 80	0 144		
Panicles per plant												
Ppl6 1	6	RM3 CDO78	1	rufipogon	20 79	0 0000	4 36	0 079	4 54	0 076	5 25	0 183
Percentage of sterility												
Ste10 1	10	CDO98 RM304	1	rufipogon	21 07	0 0000	3 55	0 076	4 29	0 090		
Ste10 2	10	RM147 RZ500	1	rufipogon	30 89	0 0000	6 37	0 108	6 22	0 128	6 56	0 131
Grains per plant												
gpl1 1	1	RZ513 RZ613	1	*rufipogon*	15 42	0 0001		0 054	3 49	0 083	**4 87**	0 112
gpl2 1	2	RG256b RM207	1	*rufipogon*	13 82	0 0002	2 95	0 049	3 44	0 074	**4 08**	0 078
gpl6 1	6	waxy RZ1002	1	Caiapo	16 85	0 0001	**3 57**	0 062	**3 73**	0 067	**7 03**	0 118
gpl11 1	11	RZ537 RZ900	1	*rufipogon*	7 50	0 0066	1 62	0 027				

1000 grain weight												
gw1 1	1	RZ613 RZ513	1	*rufipogon*	18 59	0 0000	2 03	0 064	**4 43**	0 214	**6 27**	0 174
gw1 2	1	RG462 RZ613	1	*rufipogon*	9 47	0 0023	**3 93**	0 034	**6 02**	0 220	**5 45**	0 199
gw3 1	3	RZ996 RM227	1	*rufipogon*	18 21	0 0000	**3 85**	0 063	**4 63**	0 116		
gw11 1	11	RZ537 RZ900	1	*rufipogon*	31 41	0 0000	**6 50**	0 104	**7 19**	0 118	**7 21**	0 121
	11	RM254 RM224	1	*rufipogon*	17 00	0 0001	3 60	0 063			**6 95**	0 092
Yield per plant												
yld1 1	1	RZ513 RZ613	1	*rufipogon*	18 39	0 0000	**3 89**	0 064	**4 09**	0 139	**5 01**	0 143
yld11 1	11	RZ537 RZ900	1	*rufipogon*	15 42	0 0001	3 28	0 054	3 44	0 070	**3 71**	

Significance thresholds levels (SPA = 3 6 IM = 3 75 CIM = 3 8) were determined by permutation tests

[b] Bold numbers indicate QTLs detected with LOD scores greater than the significance threshold levels A normal font indicates QTL peaks in the same regions that were detected in the alternate experiment or alternate analyses with LOD scores below the significance threshold levels

Days to Heading

Four QTLs were significantly associated with days to heading *O rufipogon* alleles contributed earliness at one of them *dth 3 2* located on chromosome 3 while for the other three *O rufipogon* alleles increased the number of days to heading The variation explained by these individual QTLs ranged from 6% to 14% as determined by CIM and was similar based on maximum likelihood estimates for SPA and IM (Table 2)

Plant height

Of the six QTLs associated with plant height that were detected by SPA and IM only *ph1 2* and *ph2 1* were detected by CIM *ph1 2* on chromosome 1 had the highest level of significance of any QTL detected in this experiment and alone accounted for 17–21% of the variation for plant height

Panicle length

No QTLs were detected for panicle length in this study

Panicles per plant

Two QTLs *ppl6 1* and *ppl11 1* affected the number of panicles per plant and they were identified as significant by all three analytical procedures In both cases the wild allele conferred a positive effect increasing the number of panicles per plant as would be predicted from the phenotype of *O rufipogon* The QTL *ppl6 1* was significant only in Experiment 1 explaining 18 3% of the phenotypic variation as determined by CIM The QTL *ppl11 1* was significant only in Experiment 2 (data not shown) and had an R^2 value of 8 5% as determined by CIM

Percent sterility

Two QTLs both located on chromosome 10 were associated with plant sterility and in both cases increased sterility was associated with the *O rufipogon* allele Only one of the QTLs *ste10 2* was detected by CIM and it explained 13% of the phenotypic variation

Grains per plant

Four regions were associated with the number of grains per plant All four were detected by both IM and CIM and two were also detected by SPA Three of the QTLs *gpl1 1* *gpl2 1* and *gpl11 1* showed the positive effect coming from the *O rufipogon* introgression The phenotypic variance explained by any of the loci associated with this trait ranged from 6 to 12% (IM and CIM)

1000 grain weight (grams)

Five QTLs were associated with 1000 grain weight In contrast to what would be predicted based on the phenotypes of the parents alleles derived from *O rufipogon* increased 1000 grain weight at all five loci The phenotypic variation explained by individual QTLs ranged from 5 to 22% (IM and CIM) The largest QTLs *(gw1 1*and *gw1* 2 located on chromosome 1) individually explained 17–22% of the variation (IM and CIM) under monoculture conditions

Yield per plant

Two genomic regions were significantly associated with yield per plant The *O rufipogon* allele was responsible for increasing yield in both cases Individual loci explained 7–14% (IM and CIM) of the variance Both QTLs were detected by all three analytical procedures

Discussion

In this study we compared the findings of this set of experiments with those previously reported by other re searchers evaluating similar characters in different cross combinations and different environments The use of a common set of molecular markers made it possible to determine whether QTLs from all the different studies were in similar regions of the rice genome By doing so newly reported QTLs could be compared to previously reported QTLs lending legitimacy to those with a prior history and suggesting caution for those flagged for the first time

Of the four QTLs identified for days to heading *dth3 1* and *dth7 1* are in the same or similar regions as previously identified QTLs The QTL *dth3 1* in this study is in a similar location as *dth3* (Xiao et al 1996) QHd3a (Li et al 1995) DH 3 (Price et al 1997) and hd3 (Lin et al 1995) and *dth7 1* coincides with Hd 4 (Yano et al 1997) Future work aimed at cloning and characterizing these alleles will confirm whether these previously reported QTLs correspond to the same or tightly linked QTLs and whether the alleles coming from *O rufipogon* are the same as those found in the cultivated *O sativa* gene pool

Plant height QTLs in the same region as *ph1 1* have been previously reported by Xiao et al (1998) and Wu et al (1996) and a QTL similar to *ph2 1* was reported by Li et al (1995) QTL *ph1 1* may represent the semi dwarf locus *sd* 1 which is located in a similar region on chromosome 1 (Cho et al 1994) The QTL *ph2 1* is in similar position to the known dwarfing mutants *d 30* (waisei shirasasa dwarf) and *d 5* (bunketsu waito) (Kinoshita 1995)

A total of 53 semi dwarf genetic stocks have been reported in rice and while nine of them are known to be allelic to the highly mutable *sd 1* locus (Rutger 1992) many others appear to be independent It will be interesting to determine how many of them define QTLs associated with plant height and to determine whether *O rufipogon* and other wild species may harbor novel

alleles that differ in structure and function from any of the alleles that have been so widely used to alter plant stature harvest index and other important agronomic characteristics in plant improvement to date

Neither of the two QTLs affecting the number of panicles per plant in this study coincided with any previously published QTLs in rice Despite the lack of historical support for the existence of these QTLs this is the only trait where the putative QTLs were supported by all three analytical procedures

Two QTLs were associated with plant sterility in this study and in both cases the *O rufipogon* alleles were associated with increased sterility A known fertility restoration locus *Rf* 1 resides on chromosome 10 in a similar position to *ste10 1* on chromosome 10 (Yao et al 1997 Tan et al 1998)

O rufipogon alleles were associated with an increase in the number of grains per plant for three of the four QTLs identified for this trait Two of these loci *gpl1 1* and *gpl2* 1 are in the same locations on chromosomes 1 and 2 as the yield QTLs reported by Xiao et al (1998) where the *O rufipogon* alleles increased yield in a hybrid variety grown under high input conditions This suggests that *O rufipogon* introgressions at these loci may be advantageous in divergent genetic backgrounds and growing environments The QTL on chromosome 1 *gpl1 1* was also located in the same region as the height QTL *ph1* 1 possibly corresponding to the *sd 1* gene It has long been observed that the *sd 1* gene not only dwarfed plant stature but also was associated with increased harvest index and yield It will be worth investigating further to understand the molecular structure and function of *O rufipogon* derived QTL in this region to better interpret the relationship between tall stature and grains per plant It should be kept in mind that the optimal plant height varies with the ecosystem in which the cultivars are grown with intermediate to tall stature being preferred under upland conditions

For all grain weight QTLs *O rufipogon* alleles were associated with heavier grains despite the fact that *O rufipogon* itself has small light grains *gw1 1* appears to be located in a similar position as 1000G (Wu et al 1996) gwt1 (Zhuang et al 1997) and g370 (Lu et al 1997) while *gw3 1* in this study appears to correspond with the position of g1318 (Lu et al 1997)

Alleles from *O rufipogon* were associated with a positive effect on yield at both putative QTL loci *yld1 1* and *yld11* 1 Both of these QTLs are in similar positions to the yield QTLs reported previously by Lin et al (1996) and Yu et al (1997) The QTL on chromosome 1 *yld1* 1 is in a similar position to the one reported by Xiao et al (1998) using the same donor species in a Chinese hybrid background grown under irrigated conditions Both of the other studies used completely different genetic material but both also evaluated yield under height input irrigated conditions Thus it will be of interest to determine whether the *O rufipogon* alleles associated

with improved yields of Caiapo under dry land conditions are the same as or different from alleles from this and other sources described previously

A total of 25 QTLs were detected for the eight traits evaluated in this study For 14 (56%) of them alleles from *O rufipogon* were associated with a positive effect on plant performance So although the *O rufipogon* parent was phenotypically inferior for seven of the eight traits studied transgressive segregants that out performed the elite Caiapo parent were obtained No strongly deleterious characters appear to be associated with any of the favorable *O rufipogon* derived QTLs described above In some cases loci associated with yield and yield components were located in the same regions as QTLs for plant height However intermediate to tall stature is considered advantageous in upland cultivars like Caiapo so until the relationship between these two phenotypes is better understood and the interaction among genes in a given genetic background can be verified no conclusions about the agronomic value of these plant height loci can be reached

Several genetic regions were associated with more than one trait indicating linkage and/or pleiotropic effects For example on chromosome 1 there are QTLs for grain weight the number of grains per plant and plant yield for which the flanking markers were the same By developing further generations of near isogenic lines containing finely mapped QTLs we aim to develop the basis for better characterizing these loci

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- **Identification of Genes from Wild Germplasm for the Improvement of Cultivated Rice The Case of Irrigated Rice in LAC**

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Introduction

The domestication process artificial selection and intensive breeding of crop varieties by man narrowed down the genetic base in many crops (Tanksley and Nelson 1996) problem that is more critical in self pollinating crops like rice (Wang et al 1992) This reduced genetic variation renders modern crop varieties more vulnerable to biotic and abiotic stresses and could explain the already observed slower rate of genetic progress achieved by plant breeding programs in several important food crops (Tanksley and Nelson 1996)

According to Sanint and Wood (1998) significant increases in rice production occurred over the past three decades in LAC nearly 300 varieties were released 90% of them targeted to irrigated rice environments Modern semi dwarf varieties account for 93% of all irrigated rice production representing more than 80% of total rice production in LAC Average yields in the irrigated areas increased from 3 3 ton/ha in the mid 1960s to 4 6 ton/ha in 1995 whilst total rice production doubled between 1967 and 1995 to reached about 20 millions ton of paddy rice Nevertheless several studies (Martinez et al 1995 FEDEARROZ 1993) have indicated that after

the initial impact obtained by the release and adoption by farmers of modern improved varieties yield per se of irrigated rice in LAC has reached a plateau in several countries including Colombia, Brazil Ecuador Dominican Republic and Uruguay It has been suggested (Cuevas Pérez 1992 Rangel and Neves 1997) that the genetic core of irrigated rice in LAC is narrow and may have reached its limits of usefulness which underlines the need to broaden the genetic base to increase rice production and the resilience of the rice based cropping systems

The Oryza wild species represent a potential source of new alleles for improving the yield quality and stress resistance of cultivated rice but they have rarely been used so far for the genetic improvement of quantitative traits (Xiao et al 1998) This is so because the superior trait of interest can not be identified phenotypically in the wild accession unless molecular markers and maps are used to identify the trait enhancing genes (Xiao et al 1998 Tanskley 1993 McCouch and Doerge 1995) Frey et al (1981) were the first to recognized that in spite of their overall inferior agronomic performance wild and weedy species could contribute to the yield increase in modern crop varieties Tanskley et al (1996) working on tomato demonstrated that molecular genetic maps could be utilized to efficiently exploit the genetic potential of wild species for the improvement of elite processing tomatoes Subsequently Tanskley and Nelson (1996) used a novel strategy referred to as advanced backcrossing QTL analysis (AB QTL) to simultaneously discovered and transferred valuable QTLs from wild germplasm to elite tomato breeding lines

Xiao et al (1998) Xiao et al (1996) and Moncada et al (2001) reported that exotic rice species like O rufipogon possessed trait improving quantitative trait loci (QTL) alleles and that the innovative use of molecular markers and maps can facilitate rice breeders the use and exploitation of wild germplasm In this study a BC_2F_2 population was developed from a cross between an elite modern variety (Bg90 2) used as a recurrent parent and an accession of O rufipogon (donor) and evaluated under irrigated transplanted conditions We were interested to determine whether O rufipogon derived QTLs would be associated with positive or negative effects under these growing conditions and compare the putative QTL containing regions with those previously reported for favorable rice growing environments

Materials and Methods

Plant material

Bg90 2 was used as the female recurrent parent in this study Bg90 2 is a modern semi dwarf variety derived from the cross IR252/Remadja introduced to Colombia in the late 70 s from Sri Lanka It has been used in the crossing program at the International Center for Tropical Agriculture (CIAT) in about 10% of the 15304 crosses made since 1977 It has a high yield potential short and strong stem medium bold grain type with high amylose content and poor milling It is susceptible to rice hoja blanca virus (RHBV) disease and to some lineages of the

rice blast fungus (*P oryzae*) Bg90 2 is one of the leading varieties in West Africa where it is grown commercially under irrigated lowland conditions *Oryza rufipogon* (Acc 105491) is from Malaysia and as species is considered ancestral to *O sativa* and has been reported (Morishima et al 1962) to be adapted to deep water as well as to drought conditions This characteristic made it especially interesting for AB QTL studies in both irrigated and upland environments

Population development

A single plant of *O rufipogon* served as the female parent in the single cross which was obtained and grown in the greenhouse at CIAT in 1994 Hybrid fertility was very high and 3 F_1 plants were used to pollinate one plant of Bg90 2 used as the female recurrent parent The resulting 153 BC_1F_1 plants were initially grown in the greenhouse and transplanted (30x50 cm) later on under irrigated conditions and evaluated based on phenotype Negative phenotypic selection for undesirable agronomic traits (spreading plants excessive shattering long awn dark color grains high sterility etc) was used to narrow the selection down to the best 42 individuals Each selected BC_1 individual was backcrossed again to Bg90 2 and 30 BC_2F_1 seeds per cross combination were sown in wooden trays in the greenhouse and later on transplanted (30x40 cm) under irrigated conditions A total of 300 BC_2F_1 plants were harvested to generate 300 BC_2F_2 families for further evaluation

Field trials (BC_2F_2 families) and trait evaluation

The 288 BC_2F_2 families along with Bg90 2 and the *O rufipogon* accession were evaluated under irrigated conditions from June to November/96 at CIAT in Palmira Data on 12 agronomic traits including grain yield/hectare (yld) 1000 grain weight (gw) panicle number/ plant (ppl) grains per panicle (gpp) total grain weight/plant (grpl) grain length (gl) panicle length (pl) grains per plant (gpl) percent sterility (ps) days to heading (dth) plant height ph) and white center (whc) were taken on 10 randomly selected plants Nomenclature for QTLs affecting traits evaluated was as described by McCouch et al (1997) Grain yield (kg/ha) of BC_2F_2 families and its progenitors was derived based on grain harvest of 40 competitive plants (20 plants/row x 2 row) and averaged over two replications

Trait correlations

Correlation among traits was evaluated by running a correlation analysis in SAS to estimate Pearson correlation coefficients

Molecular Characterization and Genotype Determination

DNA Extractions and RFLP Assays

DNA of young leaves from the parental genotypes and segregating population was extracted and PCR assay were made Five restriction enzymes EcoRI EcoRV DraI HindIII and XbaI were used both in the initial parental RFLP survey and in assaying the BC_2F_2 population A total of 127 markers (83 RFLPs and 44 SSRs) were used to analyze the BC_2F_2 progeny

SSR Assays PCR Amplification and Non Radioactive Detection

The microsatellites pair primers were tested for PCR amplification and polymorphism using DNA from the parents A total of 44 SSRs were tested in the progeny using Sequi Gen equipment (BIO RAD) serie No 132BR79933 After electrophoresis the bands were revealed using a silver staining procedure

QTL mapping

Linkage analysis in the BC_2F_2 population was performed using Mapmaker/Exp Version 3 0b (Lander et al 1987) The order of the RFLPs and SSRs was assigned according to the interspecific mapping population described in Causse et al (1994) and Chen et al (1997) respectively Minimal changes were made in the order of the markers with the ripple command

QTL Analysis

The twelve quantitative traits for yield and yield related characters were associated with the 127 molecular markers using simple regression analysis of Qgene (Nelson 2000) The proportion of phenotypic variance attributable to a particular QTL was estimated by the coefficient of determination (R^2)

Results

Polymorphism of Markers

Sixty percent of the 150 RFLPs and 80 % of the SSRs were polymorphic in the parents Bg90 2 and O *rufipogon* These results are very similar to the ones reported by Moncada et al (2001) The BC_2F_2 population was evaluated using 83 RFLPs and 44 SSTs The SSRs were used to fill gaps after the RFLPs were mapped No homozygous genotypes with respect to the donor parent were found while both homozygous and heterozygous were found with respect to the recurrent parent This could be explained by the strict negative selection applied in the BC_1F_1 and BC_2F_1 generations

Marker Segregation

In an unselected BC_2 population the expected segregation ratio would be 75% homozygous (Bg90 2/Bg90 2) 25% heterozygous (Bg90 2/O *rufipogon*) resulting in an allele frequency of 87 5 Bg90 2 12 5 O *rufipogon* alleles (Moncada et al 2001) A different situation was found in this study A chi square test for goodness of fit was run to determine the segregation ratio for each of the 127 markers with regard to the expected ratio Only 25 2% of the markers followed the expected ratio while 16 5% of them showed that less than 12 5% of the genes came from O *rufipogon* 58 3% of the markers showed more than 12 5% These results indicate a significant deviation from the expected ratio (87 5 12 5) presumably due in large part by the selection imposed in the BC_1 and BC_2 generations Our data show that the percentage of alleles from the donor parent increased in spite of the negative selection applied suggesting that the phenotype is not a good indicator of the genotype as indicated by Tanksley and McCouch (1997) A similar situation was also reported by Moncada et al (2001)

Trait Correlations

Correlations among traits were evaluated at P=0 05 and 0 01 as summarized in Table 3 For the majority of variables the degree of the correlation was medium to low and no strong correlations were found The strongest correlation was found between grains per plant and grains per panicle (0 67) and between panicle length and plant height (0 50)

Genetic Variation for the Different Traits

As illustrated in Figure 1 positive transgressive segregation in the BC_2F_2 population was observed for all traits However few families had greater number of panicle/plant compared to

O rufipogon The transgressive segregation observed suggested positive genotype x genotype interaction where O *rufipogon* alleles increased performance in a largely Bg90 2 genetic background For example in the case of grain yield/hectare 16% of the BC_2F_2 families yielded up to 26% more than Bg90 2 Some of these families had lower number of panicles/plant compared to Bg90 2 while other had a higher 1000 grain weight compared to Bg90 2 Approximately 48% of the families had higher total grain weight/plant than Bg90 2 44% showed increased panicle length and 28% of them had increased grain length

Putative QTL Regions

Molecular markers associated with grain yield and its components as well as simple regression analysis is shown in Table 4 Significant QTL regions were detected for all traits measured at the 5 level of significance The R2 values for the majority of markers were very low with the exception of marker RZ538 associated with panicle length and plant height and RZ730 Several markers were associated with several traits Based on single regression analysis the following QTL regions were found

Grain yield/hectare (yld)

Thirteen markers were found associated with QTLs affecting grain yield/ha in chromosomes 2 3 5 6 9 10 and 12 Molecular markers RM13 and RM215 on chromosomes 5 and 9 respectively were associated with alleles derived from O *rufipogon* affecting grain yield in a positive way

1000-grain weight (gw)

Nine markers were associated with the weight of 1000 grains at P=0 05 located on chromosomes 1 4 5 6 8 11 and 12

Panicle number/plant (ppl)

Eight markers located on chromosomes 2 3 6 8 11 and 12 determined numbers of panicles per plant

Grains per panicle (gpp)

Eleven markers on chromosomes 1 2 3 4 and 5 were found associated with QTLs affecting number of grains per panicle at P=0 05

Total grain weight per plant (grpl)

Two genomic regions on chromosome 1 and 9 were found associated with this trait at P=0 05

Grain Length (gl)

Twenty one markers located on chromosomes 1 2 3 4 5 6 7 9 10 11 and 12were found associated with grain length at P=0 05

Panicle Length (pl)

Nineteen genomic regions on chromosomes 1 3 4 5 6 7 8 9 11 and12 were found significant at P=0 05

Grains per Plant (gpl)

Seventeen markers on chromosomes 1 2 3 5 6 7 and 12 were found significant at P=0 05

Percent Sterility (ps)

Fifteen genomic regions located on chromosomes 1 2 3 6 7 9 10 and 12 were associated with this trait at P=0 05

Days to Heading (dth)

Ten genomic regions located on chromosomes 1 6 7 8 11 and 12 were associated with alleles affecting days to flowering

Plant Height (ph)

Twenty nine markers located on chromosomes 1 2 3 4 5 6 7 9 10 11 and 12 were associated with plant height at P=0 05 These alleles increased plant height in relation to Bg90 2 Markers RZ538 and RZ730 had a high R2 value

White Center (WHC)

Ten markers located on chromosomes 3 5 6 7 and 11 were associated with QTLs affecting the expression of white center in the rice endosperm

Molecular map

Mapmaker version 3 0 (Lander et al 1987) was used to construct the molecular map for the BC_2F_2 population showing the location of markers associated with putative QTLs affecting grain yield and its components (Figure 2)

Discussion

A marker assisted advanced backcross strategy was used to identify QTLs associated with twelve agronomic traits in a BC_2F_2 population derived from an interspecific cross between Bg90 2 an irrigated *O sativa* subspecies indica rice cultivar and an accession of O *rufipogon* (IRGC 105491) from Malaysia Simple regression analysis was used to identify genomic regions associated with selected traits A more complete analysis is still underway Results suggest that *O rufipogon* may possess significant putative QTLs associated with the 12 traits studied at the 5% level of significance Nevertheless we have to keep in mind and perspective that QTLs studies are confounded by both genetic and environmental interactions that make it difficult to assess and predict which putative QTLs are likely to be more stable and consistent when transferred to a new genetic background Fulton et al (1997) suggested that QTLs more consistent over environments were more likely to be useful than those that showed an extremely high significance value but were detected in only one year or under one set of conditions

Trait correlations

Alleles from O *rufipogon* seemed to be associated with a positive effect on grain yield These results agree with previous studies (Lin et al 1996 Yu et al 1997 Xiao et al 1998 and Moncada et al 2001) The positive yield effect reported by Xiao et al (1998) due to the wild QTL derived from *O rufipogon* appears to have a high value Our report is the first one using as a recurrent parent an indica cultivar in a tropical irrigated environment On the other hand it is noteworthy to highlight that the picture that is emerging when we consider data from these different groups is that in O *rufipogon* possess alleles that affect grain yield and yield components in a positive manner and they are likely to be expressed regardless of environments location and genetic background Martinez et al (2000) showed that some breeding lines derived from this population and selected based on phenotype kept their yield advantage over the recurrent parent (Bg90 2) through the F_5 generation This suggests that yield advantage observed as early as in the BC_2F_2 generation was a real one not due to hybrid vigor and passed on through different cycles of phenotypic selection

Breeding for high yield potential for a given environment is a very important objective in almost every varietal improvement program Yet yield gains often comes at the cost of prolonging

growth duration (Ishizuka et al 1973) There was a significant positive but very low correlation (0 19139) between grain yield and flowering (Table 1) Xiao et al (1998) reported that QTLs yld1 1 and yld2 1 increased grain yield with no detectable effect on maturity More recently Martinez et al (2000) presented data suggesting that probably a similar case is operating in our case They showed that some advanced F_5 lines derived from a BC_2F_2 population from the Bg90 2/ O rufipogon cross yielded significantly more than Bg90 2 with a growth duration longer than *O rufipogon* but shorter than Bg90 2

On the other hand correlation between plant height and grain yield was negative but no significant (0 046) Our data (Table 4) show that two putative QTLs associated with markers RZ538 and RZ730 had a large effect on plant height Beachell and Jennings (1965) showed that there is a negative and significant correlation between plant height and yield but this was not the case in our study Our data seem to suggest that lines taller than Bg90 2 can be developed with no reduction in grain yield due to lodging

From a breeding perspective these wild QTLs could be used immediately to improve traits of agronomic importance In fact BC_2F_5 lines having either consistent yield advantage over the recurrent parent or longer and slender translucent grain type compared to both parents have been identified in our breeding nursery at CIAT and made available to national rice programs for testing under local conditions

Implications for Rice Breeding

Irrigated rice represents the most important production system for rice worldwide Major advances have been made in increasing rice production worldwide as a result of large scale adoption of modern high yielding varieties and improved cultural practices Nevertheless in order to keep up with increased rice demand it has been estimated that we will have to produce 50% more rice This increased demand will have to be met from less land with less water less labor and less chemicals (Khush 1999)

Different approaches mainly population improvement ideotype breeding heterosis breeding wide hybridization genetic engineering and molecular breeding are being explored for increasing the yield potential of rice These approaches depend on the fact that genetic variability is needed to achieve genetic gains Unfortunately the genetic base of rice (both irrigated and upland) in Latin America is narrow which may explain the yield plateau observed in several sites in this region

Base broadening (Simmonds 1993) or crop gene pool enrichment (Frey 1999) has been proposed as a good alternative to transfer useful genes from un adapted germplasm into populations that are useful for developing new cultivars Our results and that of Moncada et al

(2000) and Xiao et al (1998) indicated that O *rufipogon* possesses new unrelated alleles to those previously used by rice breeders with positive effects on traits of economic importance such as grain yield and yield components Therefore these alleles represent new sources of genetic variability that will help breeders diversify the genetic reservoir available for crop improvement Rice breeders will have new alleles to broaden the genetic base of cultivated rice while at the same time explore opportunities for new gene recombination for the improvement of traits of economic importance New gene combinations may result in better rice varieties

By targeting wild germplasm for use in population improvement alleles that were left behind during the domestication process or those that are unique to specific gene pools can be introgressed into elite germplasm (Tanskley and McCouch 1997) Therefore new possibilities for increasing the yield potential of irrigated rice can be explored By using molecular maps and markers trait improving QTLs can be selectively transferred and pyramided onto modern rice varieties

Our data and that of others (Moncada et al 2000 Xiao et al 1998 Xiao et al 1996) not only confirmed earlier reports (Morishima 1962 Schmit 1997) about the plasticity of O *rufipogon in* terms of adaptation to different environments but it demonstrated for the first time that *O rufipogon* can contribute to yield increase in both upland and irrigated environments ranging from the highly favored transplanted rice to drought stress upland rice These results also indicate that QTLs from O *rufipogon* have positive effects on grain yield in different genetic backgrounds including both temperate and tropical japonica and indica rice

In rice most of the time new progenitors have been selected based on pedigree geographic origin and/ or for belonging to a given race group (indica, japonica javanica) Many elite breeding lines produced by international rice research centers (IRRI CIAT IITA WARDA) and other rice improvement programs around the world were obtained from crosses between parents that go back to a limited number of land races (Hardgrove 1980 Cuevas Perez et al 1992) This situation suggests that a re shuffling of alleles has been going on for several decades and underscores the need to broaden the genetic base of cultivated rice and select new progenitors based on genotype rather than on phenotype Our results indicate that new alleles with positive effects on several agronomic traits are now available to broaden and enhance the genetic base of rice in Latin America

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Table 3 Correlation Coefficients among Traits in an *O rufipogon* derived BC_2 Population (*=p 0 05,=p0 01)**

	YLD		GW		PPL		GPP		GRPL		GL		PL		GPL		PS		DTH
Grain yield (yld)																			
1000 grain weight (gw)	0 01																		
Panicle number per plant (ppl)	0 11		0 04																
Grains per panicle (gpp)	0 27	**	0 15		0 16														
Total grain weight per plant (grpl)	0 00		0 26	**	0 11		0 13	*											
Grain length (gl)	0 03		0 26		0 01		0 04		0 06										
Panicle length (pl)	0 12		0 25		0 05		0 36		0 20	*	0 28								
Grains per plant (gpl)	0 33	*	0 02		0 37		0 67		0 22	*	0 02		0 31	**					
Percent sterility (ps)	0 10		0 02		0 01		0 04		0 06		0 31	*	0 03		0 13				
Days to heading (dth)	0 17	*	0 12		0 02		0 11		0 02		0 01		0 21		0 04		0 11		
Plant height (ph)	0 04		0 04		0 04		0 11		0 10		0 13	*	0 50	**	0 18	**	0 20	*	0 13

Table 4 Molecular Markers Associated with Putative QTLs Affecting Yield and Yield Components in the BC_2F_2 Population Simple Regression Analysis (SAS)

Chr	Marker	Quantitative Traits	N	MSE	R^2	B0		B1	
2	CDO524	YLD	242 0	1146898 5	0 0161	4317 5	**	367 8	**
3	RG100	YLD	241 0	1101650 0	0 0261	4347 2	**	384 0	**
5	RM13	YLD	232 0	1078071 0	0 0350	5843 0	**	-472 1	**
6	RM204	YLD	234 0	1135434 4	0 0264	4126 8	**	469 0	**
6	RM217	YLD	224 0	1070675 0	0 0487	3135 4	*	963 0	*
9	RM215	YLD	229 0	1067606 7	0 0274	5707 6	**	390 9	**
9	RM242	YLD	217 0	1143579 3	0 0231	5623 6	*	352 8	*
10	RM222	YLD	224 0	1130783 7	0 0232	4259 4	**	418 3	**
12	G1391	YLD	236 0	1141902 1	0 0341	4160 5	**	472 9	**
12	RG543	YLD	243 0	1119267 8	0 0359	3986 5	**	547 9	**
12	RG574	YLD	235 0	1160099 8	0 0207	4190 4	**	432 8	**
12	RG901	YLD	242 0	1099610 8	0 0427	3535 1	**	767 6	**
12	RZ816	YLD	243 0	1125099 4	0 0308	3738 3	**	656 6	**
1	RG957	GW	240 0	5 1	0 0195	29 1	**	0 8	**
1	RZ538	GW	222 0	5 7	0 0274	29 5	**	1 1	**
4	RZ590	GW	238 0	5 5	0 0172	26 1	*	0 8	**
5	RM164	GW	238 0	5 2	0 0811	23 8	**	2 0	*
5	RM249	GW	239 0	5 8	0 0177	26 2	**	0 7	**
6	RZ1002	GW	238 0	5 3	0 0216	31 3	**	1 9	**
8	RG598	GW	242 0	5 4	0 0192	30 9	**	1 7	**
8	RZ323	GW	240 0	5 4	0 0219	31 3	**	1 9	**
12	G1112	GW	238 0	5 2	0 0478	25 1	**	1 3	**
2	RM48	PPL	234 0	12 4	0 0169	13 6	**	1 0	**
3	RM55	PPL	227 0	12 8	0 0171	18 4	**	1 7	**
6	RG445	PPL	238 0	12 5	0 0171	17 4	**	1 2	**
8	RM44	PPL	242 0	12 0	0 0306	4 1	NS	5 5	**
11	RZ536	PPL	222 0	12 5	0 0205	17 9	**	1 5	**
12	G1112	PPL	238 0	12 5	0 0185	17 6	**	1 3	**
12	RG574	PPL	235 0	12 1	0 0357	18 6	**	1 9	**
12	RZ816	PPL	243 0	12 4	0 0175	18 3		1 6	**
1	RM1	GPP	225 0	696 4	0 0398	94 3	**	15 7	**
1	RM220	GPP	219 0	730 7	0 0202	102 8	**	11 1	**
1	RZ538	GPP	222 0	720 3	0 0262	102 3	**	11 8	**
2	RZ476	GPP	223 0	710 2	0 0317	101 4	**	12 4	
2	RZ599	GPP	241 0	699 1	0 0355	95 7	*	15 1	**
3	RG369	GPP	239 0	713 8	0 0216	108 1	**	9 0	
3	RG913	GPP	239 0	701 5	0 0211	109 0	**	8 7	**
4	RG476	GPP	210 0	736 0	0 0210	106 0	**	10 2	
4	RZ590	GPP	238 0	685 8	0 0430	97 7	**	14 4	**
5	RM164	GPP	238 0	668 6	0 0749	84 5	**	21 2	*
5	RM31	GPP	231 0	716 3	0 0365	94 7	**	15 2	**
1	RG957	GRPL	240 0	135 1	0 0163	50 9	*	-4 0	**
9	RM215	GRPL	229 0	131 1	0 0239	50 3	**	-4 0	
1	RG957	GL	240 0	0 1	0 0205	9 2	**	0 1	**
1	RZ538	GL	222 0	0 1	0 0318	9 2	**	0 1	**
2	RZ476	GL	223 0	0 1	0 0252	9 2	**	0 1	**

Chr	Marker	Quantitative Traits	N	MSE	R^2	B0		B1	
3	RM251	GL	236 0	0 1	0 0521	8 7	**	0 2	
3	RM55	GL	227 0	0 1	0 0226	8 7	**	0 2	*
3	RZ630	GL	242 0	0 1	0 0211	9 1	**	0 1	**
4	RM261	GL	231 0	0 1	0 0348	8 7	**	0 1	**
4	RZ590	GL	238 0	0 1	0 0185	8 8	**	0 1	*
4	RZ69	GL	238 0	0 1	0 0814	8 5	**	0 3	
5	RM122	GL	236 0	0 1	0 0605	9 3		0 2	
5	RZ225	GL	232 0	0 1	0 0202	9 1		0 1	
5	RZ67	GL	226 0	0 1	0 0245	8 8	**	0 1	**
6	RM204	GL	234 0	0 1	0 0257	8 7	**	0 1	**
6	RZ667	GL	242 0	0 1	0 0208	8 6	**	0 2	**
7	RM234	GL	234 0	0 1	0 0401	9 3	*	0 2	**
9	RM242	GL	217 0	0 1	0 0263	9 2	**	0 1	**
10	RM216	GL	215 0	0 1	0 0364	8 7	**	0 2	**
11	BCD808	GL	242 0	0 1	0 0279	8 6	**	0 2	
11	G1465	GL	229 0	0 1	0 0281	8 7	**	0 1	*
12	G1112	GL	238 0	0 1	0 0301	8 7	**	0 1	**
12	RG190	GL	238 0	0 1	0 0561	8 6	**	0 2	
1	RG957	PL	240 0	1 3	0 0229	24 7	**	0 5	
1	RM1	PL	225 0	1 2	0 0210	22 9	**	0 5	
1	RZ538	PL	222 0	1 0	0 2145	26 4	**	1 4	
1	RZ730	PL	233 0	1 2	0 1029	25 9	**	1 1	**
3	RG913	PL	239 0	1 3	0 0183	23 2	**	0 3	**
3	RM55	PL	227 0	1 3	0 0212	22 7	**	0 6	**
4	RG476	PL	210 0	1 4	0 0226	23 1	*	0 5	**
4	RZ590	PL	238 0	1 3	0 0509	22 6	*	0 7	**
5	RM31	PL	231 0	1 2	0 0426	22 5	**	0 7	**
6	RM3	PL	236 0	1 3	0 0438	25 4	**	0 8	**
7	RZ214	PL	243 0	1 3	0 0359	22 7	**	0 6	**
8	RZ617	PL	236 0	1 3	0 0237	23 3	**	0 4	*
9	RM215	PL	229 0	1 2	0 0814	25 1	**	0 7	**
9	RM242	PL	217 0	1 2	0 0477	24 7	*	0 5	**
11	RM224	PL	219 0	1 3	0 0292	22 9	**	0 5	
12	G1112	PL	238 0	1 3	0 0506	22 6	**	0 7	**
12	RG574	PL	235 0	1 3	0 0197	23 0	**	0 5	
12	RG9	PL	234 0	1 3	0 0460	23 0	**	0 5	**
12	RZ816	PL	243 0	1 3	0 0287	22 5	**	0 7	**
1	RM1	GPL	225 0	142453 2	0 0300	1635 7	**	194 3	**
1	RM220	GPL	219 0	139836 2	0 0257	1669 4	**	173 3	
1	RZ538	GPL	222 0	135868 9	0 0679	1506 2	**	265 7	
1	RZ730	GPL	233 0	139764 0	0 0220	1692 1	**	166 8	*
2	RM48	GPL	234 0	135945 9	0 0192	1814 9		112 8	
2	RZ476	GPL	223 0	138735 4	0 0373	1654 0	**	188 5	**
2	RZ599	GPL	241 0	138501 3	0 0299	1635 5	**	194 4	**
3	RG100	GPL	241 0	139335 7	0 0229	1781 9	**	127 5	**
5	RM164	GPL	238 0	136590 3	0 0368	1613 9	**	208 1	*
5	RM31	GPL	231 0	140120 6	0 0378	1591 0	**	216 7	**
6	RM217	GPL	224 0	137423 9	0 0188	1601 1	*	211 1	*
7	RG30	GPL	237 0	139964 3	0 0264	1625 4		199 8	

Chr	Marker	Quantitative Traits	N	MSE	R^2	B0		B1	
12	G1391	GPL	236 0	140923 7	0 0243	1757 1	**	139 7	**
12	RG457	GPL	229 0	142126 4	0 0233	1754 1	**	141 2	**
12	RG543	GPL	243 0	140534 3	0 0166	1758 6	**	130 7	**
12	RG574	GPL	235 0	140493 7	0 0181	2252 3	**	140 7	**
12	RG869	GPL	227 0	143611 6	0 0199	1771 5	**	129 6	**
1	RM1	PS	225 0	32 9	0 0408	23 8	**	3 5	**
1	RM220	PS	219 0	35 3	0 0178	26 2	**	2 3	**
1	RZ538	PS	222 0	32 7	0 0461	24 2	**	3 4	**
1	RZ543	PS	242 0	33 0	0 0208	22 8	**	3 8	**
1	RZ730	PS	233 0	32 1	0 0375	23 9	*	3 3	**
1	RZ995	PS	230 0	33 2	0 0173	27 5	**	1 6	**
2	RM6	PS	233 0	35 1	0 0184	33 9	**	1 9	**
3	RZ545	PS	243 0	33 2	0 0219	26 6	**	2 1	**
4	RZ884	PS	243 0	33 4	0 0163	27 3	**	1 7	*
7	RM234	PS	234 0	34 4	0 0173	34 8	**	2 3	**
9	RG451	PS	239 0	33 7	0 0180	26 5	**	2 1	**
9	RM215	PS	229 0	32 8	0 0193	27 5	*	1 8	**
10	RM216	PS	215 0	34 9	0 0434	24 1	**	3 5	**
12	G1391	PS	236 0	31 2	0 0232	26 8		2 0	**
12	RG869	PS	227 0	31 8	0 0204	26 8	**	2 0	**
1	RM220	DTH	219 0	4 1	0 0215	101 0	**	0 9	**
1	RM5	DTH	233 0	4 1	0 0236	101 1	**	0 8	**
1	RZ538	DTH	222 0	4 2	0 0184	101 2	**	0 8	**
1	RZ995	DTH	230 0	4 1	0 0331	101 2	**	0 8	**
6	RM217	DTH	224 0	4 2	0 0190	100 3	**	1 2	**
7	CDO407	DTH	242 0	4 1	0 0230	100 9	**	0 9	**
8	RM44	DTH	242 0	4 1	0 0230	108 1	**	2 8	**
11	RM254	DTH	225 0	4 2	0 0186	104 6	**	1 1	**
12	G1391	DTH	236 0	4 1	0 0255	101 2	**	0 8	*
12	RG869	DTH	227 0	4 0	0 0198	101 3	**	0 7	*
1	RG957	PH	239 0	101 5	0 0667	110 6	**	7 2	**
1	RZ444	PH	241 0	106 1	0 0168	104 6	**	3 9	**
1	RZ538	PH	221 0	28 3	0 7274	139 8	**	23 2	**
1	RZ730	PH	232 0	78 8	0 2978	129 8	**	17 1	**
2	RG25	PH	241 0	106 0	0 0175	104 6	**	3 8	**
2	RM233A	PH	234 0	109 9	0 0313	104 5	**	-4 1	**
3	RM251	PH	235 0	108 8	0 0340	89 9	**	4 5	
3	RM60	PH	231 0	104 8	0 0274	88 4	**	4 8	**
4	BCD135	PH	241 0	105 3	0 0240	106 6	**	-4 9	*
4	RG177	PH	241 0	105 9	0 0184	105 2	**	-4 1	**
4	RG375	PH	239 0	106 1	0 0214	105 3	**	-4 2	**
4	RG908	PH	241 0	104 5	0 0314	107 1		5 2	
5	RG474	PH	240 0	106 3	0 0184	104 5	**	3 8	*
5	RM164	PH	237 0	108 2	0 0326	108 0	**	5 6	
5	RM249	PH	238 0	106 5	0 0354	105 0	**	-4 3	**
5	RZ225	PH	231 0	109 6	0 0187	103 2	**	3 2	**
6	RM3	PH	235 0	107 3	0 0253	108 5	**	5 7	**
6	RZ682	PH	241 0	105 2	0 0253	106 7	**	-4 9	**
7	RZ272	PH	241 0	105 2	0 0250	106 0	**	-4 6	**

Chr	Marker	Quantitative Traits	N	MSE	R^2	B0		B1	
9	RM215	PH	228 0	104 0	0 0227	103 5	*	3 5	**
10	BCD386	PH	241 0	106 1	0 0169	104 4	*	3 7	**
11	C794	PH	239 0	106 4	0 0211	105 4	**	-4 3	**
11	C82	PH	241 0	105 7	0 0202	104 9	*	-4 0	**
11	G44	PH	241 0	105 9	0 0187	105 2	**	-4 2	**
12	G1112	PH	237 0	106 4	0 0234	89 9	**	4 2	**
12	RG574	PH	234 0	107 9	0 0179	90 5	**	3 9	*
12	RG869	PH	226 0	109 6	0 0202	104 0	**	3 6	**
12	RZ397	PH	241 0	106 1	0 0165	104 4		3 7	**
12	RZ76	PH	241 0	105 7	0 0202	105 4	*	-4 3	**
3	RG100	WHC	241 0	0 6	0 0181	3 0	**	0 2	**
3	RM7	WHC	231 0	0 6	0 0432	3 5	*	0 5	**
5	RM122	WHC	236 0	0 6	0 0452	1 8		0 4	**
5	RM13	WHC	232 0	0 6	0 0167	2 1	*	0 2	**
5	RM164	WHC	238 0	0 6	0 0709	1 5	**	0 6	**
5	RM249	WHC	239 0	0 6	0 0375	2 0	**	0 3	**
6	RM3	WHC	236 0	0 6	0 0181	3 3	**	0 4	**
7	RM11	WHC	215 0	0 6	0 0302	3 3	**	0 4	**
7	RM214	WHC	236 0	0 6	0 0420	3 8	**	0 6	**
11	RG303	WHC	227 0	0 6	0 0247	3 6		0 5	**

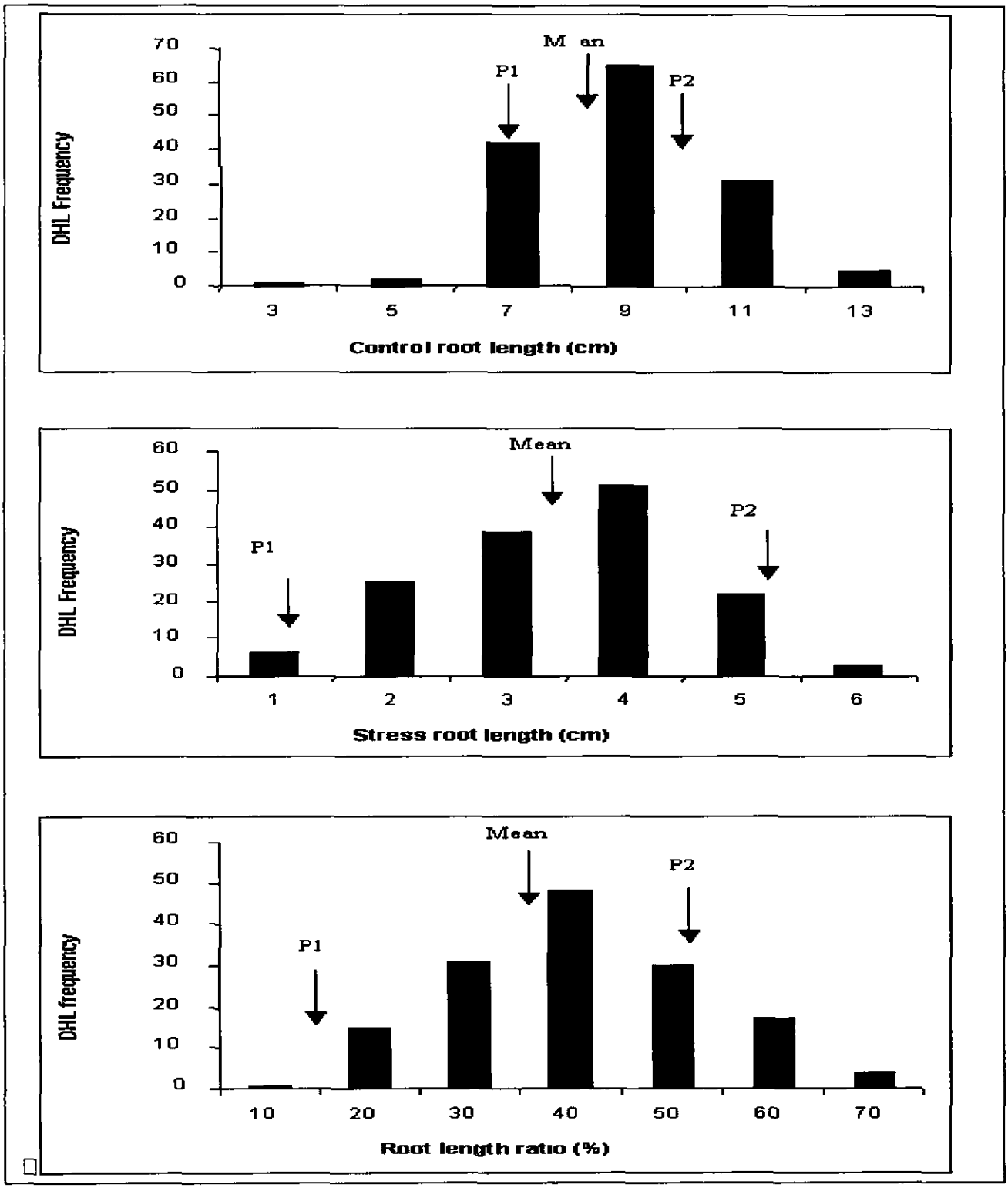

Figure 1 Frequency Distribution of Phenotypes for each Trait in the F_2BC_2 Families Derived from the Cross Bg90-2 and *O rufipogon*

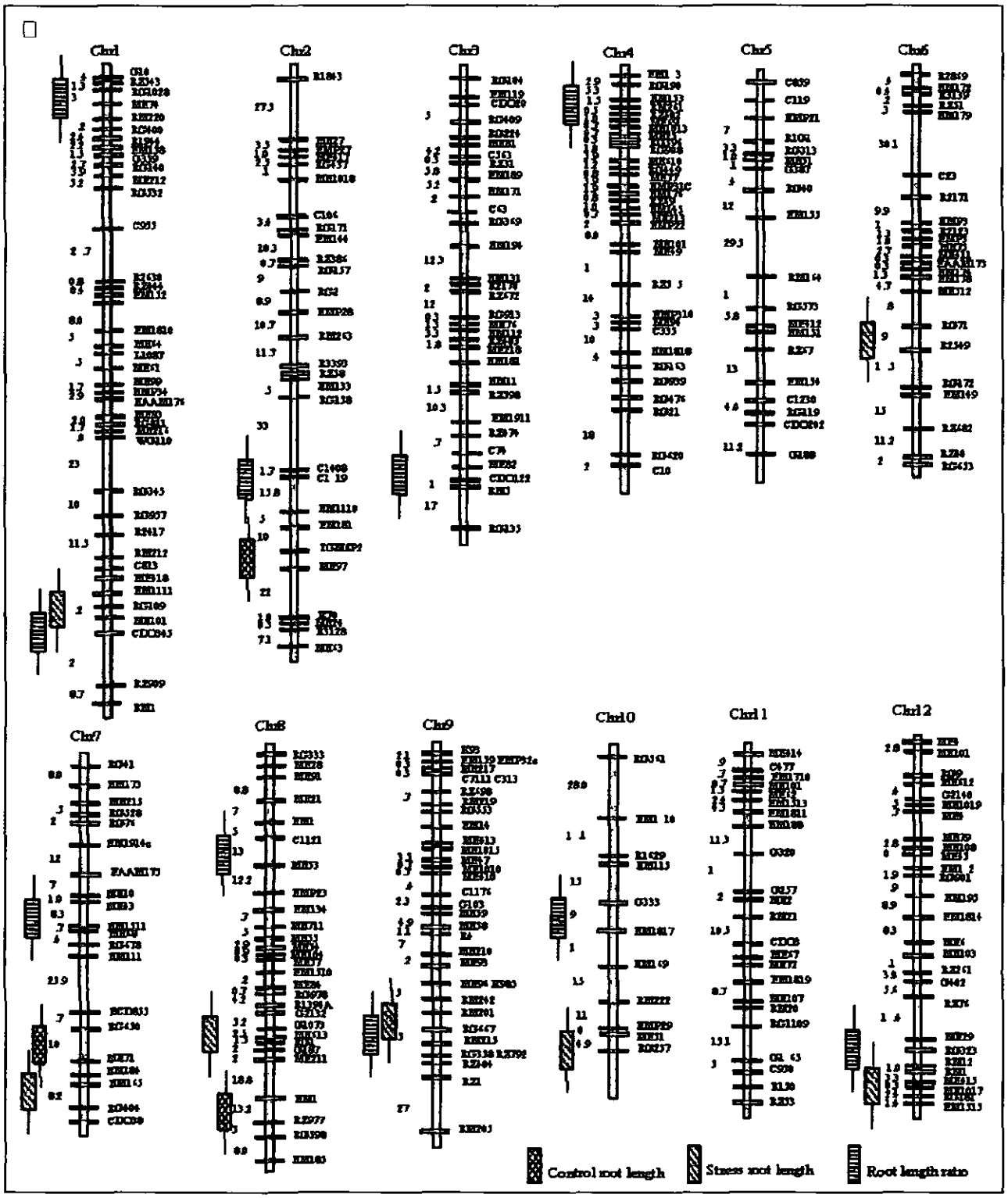

Figure 2 Putative Location of Associated with Yield and Yield Components in the Cross Bg90 2/*O. Rufipogon* based on Simple Regression Analysis

- **Mapping Genes Controlling Al Tolerance in Rice Comparing Different Genetic Backgrounds**

Nguyen Vinh T Bay D Nguyen Surapong Sarkarung Cesar Martinez Andrew H Paterson Henry T Nguyen

Abstract

Almost 40% of the world s cultivated land is classified as acidic Aluminum toxicity is the main factor limiting the productivity of crop plants in acid soil areas particularly in the tropics and subtropics In recent years intense research has been conducted on the cellular and physiological bases of Al toxicity to understand the genetic mechanisms of Al tolerance in plants and to develop molecular strategies for crop improvement In this study a doubled haploid population derived from rice breeding lines CT9993 and IR62266 (*Oryza sativa* L) was used to map genes controlling Al tolerance A genetic linkage map consisting of 315 DNA markers (RFLP AFLP and SSR) was constructed to determine the position and nature of QTLs affecting Al tolerance Three characters viz control root length (CRL) stress root length (SRL) and root length ratio (RR) were evaluated for the DH lines and the parents at the seedling stage in nutrient solution A total of 20 QTLs controlling root growth under Al stress and control conditions were detected and spread over 10 out of 12 chromosomes in the rice genome The results indicated that root growth and aluminum tolerance in rice seedlings are controlled by multiple genetic factors Two largest QTLs viz *QAlRr1a* and *QAlRr8a* for root length ratio a measurement of Al tolerance were located on chromosome 1 and 8 respectively Three other QTLs in addition to *QalRr8a* were apparently unique in the CT9993 x IR62266 mapping population which may explain the high level of Al tolerance in CT9993 Comparative mapping identified a conserved genomic region on chromosome 1 associated with Al tolerance across three rice genetic backgrounds This region provides an important starting point for isolating genes responsible for different mechanisms of aluminum tolerance and understanding the genetic nature of this trait in rice and other cereals

Introduction

Soil salinity acidity and mineral deficiencies will continue to be one of the major problems limiting crop productivity throughout the world Sanint and Woods (1998) reported that in 1995 about 45% of rice (*Oryza sativa* L) produced in Latin America was grown under upland condition The upland soils are unfertile and mostly acidic in nature Moreover approximately 500 million ha of oxisols and ultisols are presently under utilized partly because of extreme soil acidity and infertility Upland crops grown in such soils suffer from Al toxicity and Ca and P deficiencies (Howeler and Cadavid 1976) Aluminum (Al) toxicity is the most important factor limiting crop productivity in acid soils which comprise large areas of the world (Kochian 1995) particularly in the tropics and subtropics (Foy et al 1978 Foy 1984)

The major symptom of aluminum toxicity is rapid inhibition of root growth (Luttge and Clarkson 1992 Rengel 1992 Delhaize and Ryan 1995) The effect of aluminum toxicity is to arrest or slow down root growth As a result stunted or shortened roots are the primary and early symptom of aluminum toxicity Bennet et al (1987) suggested that the root cap is a site of perception of Al injury Roots injured by high Al are usually stubby and thick and become dark colored brittle poorly branched and rubberized (Foy 1983) Several techniques involving the study of root growth such as absolute root length root re growth and hematoxylin have been employed in evaluating for Al tolerance in plants (Gallego and Benito 1997 Lafever and Campbell 1978 Riede and Anderson 1996) In rice absolute root length techniques or root length ratio has been widely used as a parameter for evaluating Al tolerance (Coronel et al 1990 Khatiwada et al 1996 Wu et al 1997) It provides major advantages over other techniques including being a simple measurement and the elimination of the genetic difference in root growth under normal culture conditions (Wu et al 2000)

By comparing the response of root and shoot to Al toxicity in wheat Briggs and Taylor (1993) and Zale (1987) found that Al stress in hydroponic system affects root characteristics much more than shoot characteristics Thus the measurement of root parameter offers the best approach to selecting or screening plant genotypes for Al tolerance The physiological and biochemical mechanisms of the toxic effect of aluminum on root elongation have been extensively investigated (Foy and Flenming 1978 Horst et al 1982 Haug and Shi 1991 Matsumoto 1991 Luttge and Clarkson 1992 Lazof et al 1994) However the genetic mechanisms controlling Al tolerance in crop plants are poorly understood (Aniol and Gustafson 1984 Carver and Ownby 1995) The inheritance of Al tolerance in barley (*Horderum vulgae* L) was reported to be controlled by a single gene (Reid et al 1969 Minella and Sorrells 1992) In contrast the genetic system controlling Al tolerance in wheat (*Triticum aestivum* L) appears to be complex and composed of major and minor genes (Aniol and Gustafson 1984 Luo and Drorak 1996) In corn (*Zea mays* L) Al tolerance is believed to be governed at a single locus with a multiple allelic series (Rhue et al 1978) Major genes for Al tolerance in rye (*Secale cereale* L) which is the most Al tolerant species in cereals (Aniol and Gustafson 1984 Manyova et al 1988) are located on chromosomes 3R 4R and 6RS using wheat rye addition lines (Aniol and Gustafson 1984) Information on the genetic mechanisms controlling Al tolerance in rice is limited and it appears to be controlled by many genes (Khatiwada 1996 Wu et al 1997 Wu et al 2000 Nguyen et al 2001)

Advances in molecular marker technology have led to the development of detailed molecular linkage maps for many plant species These maps have allowed the dissection of quantitatively expressed trait into Mendelian factors referred to as quantitative trait loci (QTLs) each linked to molecular markers of known map position (Paterson et al 1988) QTL mapping could lead to application in crop improvement through marker assisted selection In addition QTL mapping and DNA markers will provide insights into comparative genetics and evolution of Al tolerance genes among cereals Al tolerance has been intensively investigated in many crop plants and molecular markers linked to genes or QTLs conferring Al tolerance have been identified in wheat (Riede and Anderson 1996) rye (Aniol and Gustafson 1984 Gallego et al 1998) maize

(Sibov et al 1999) barley (Tang et al 2000) and rice (Wu et al 2000 Nguyen et al 2001) The main objectives of this study were to map genes controlling Al tolerance in a unique upland rice germplasm and to compare QTLs for Al tolerance across different rice genetic backgrounds and other cereals

Materials and Methods

Plant Material

A total of 146 doubled haploid (DH) lines from a cross between CT9993 5 10 1 M (abbreviated as CT9993 an upland *japonica* ecotype tolerant to Al toxicity) and IR62266 42 6 2 (abbreviated as IR62266 an *indica* ecotype susceptible to Al toxicity) were taken for the present study These parents were pre screened for Al toxicity with other rice genotypes known to be Al tolerant such as Azucena (Khatiwada et al 1995) and Chiembau (Nguyen et al 2001) at different Al concentrations CT9993 was found to be the most tolerant rice genotype to Al toxicity among several lines tested (Nguyen et al 2000) CT9993 was selected under acid soil condition in the rice breeding program at CIAT Its pedigree came from complex crosses that involved varieties/cultivars which are highly tolerant to Al toxicity and low pH such as Moroberekan IRAT216 IRAT13 IRAT 120 and IRAT121 from Africa and Latin America

Aluminum Tolerance Screening

The parental lines and DH progenies were screened for Al tolerance in the laboratory using a nutrient solution culture modified after Khatiwada et al (1996) The experiment design was a randomized complete block with four replications Seeds with uniform size were sterilized with 15% H_2O_2 rinsed with distilled water and incubated on filter papers soaked with distilled water in the dark at 30°C for two days Germinated seeds were grown in distilled water for another two days in a culture room maintained at 27±2°C Seedlings were then transferred to a styrofoam sheet with a nylon net bottom with one seedling per hole and three seedlings in one row per line in each replication The styrofoam sheets were floated on a nutrient solution (Yoshida et al 1976) in a plastic tray containing either 0 (control) or 30 ppm Al (stress treatment) The pH of the solutions was adjusted daily to 4 0 with 1N NaOH or 1N HCl The hydroponic trays and seedlings were maintained in the culture room at 27±2 C with 12 hr of light at 300 PPFD The longest root of each seedling was measured after 10 days of growth in control or stress solution The ratio of average root length under stressed over control conditions for each line in each replication was used as a measurement of Al tolerance

Statistical Analysis

Standard analysis of variance (ANOVA) was performed to test the significant genetic variation among the DH lines for the three traits using SAS procedure (SAS Institute 1988) Broad sense heritabilities (h^2) were computed from the estimates of genetic (σ^2G) and residual (σ^2e) variances derived from the expected mean squares of the analysis of variances as $h^2 = (\sigma^2G / (\sigma^2G + \sigma^2e /$ k)) where k was the number of replications

Linkage Map and QTL Analysis

A genetic linkage map consisting of 315 marker loci including 145 RFLPs 153 AFLPs and 17 SSRs was constructed based on the 154 DH lines using MAPMAKER/Exp version 3 0 covered 1 788 cM in length using the Kosambi function with an average distance of 5 7 cM between adjacent markers (Zhang et al 2001) QTL analysis was performed according to the method of interval mapping (Paterson et al 1988 Lander and Bostein 1989) using MAPMAKER/QTL 1 1 (Lincoln et al 1992) Based on a chromosome number of 12 and observed map length of 1847cM a LOD score of 2 8 was selected as the threshold for declaring presence of a QTL to reduce false positive QTL at P<0 05 (Lander and Bostein 1989) Independent test was carried out when there were more than one QTLs for the same trait located on the same chromosome (Paterson et al 1988 Lander and Bostein 1989) QTLs were designed with a *Q* to indicate they were detected through QTL mapping followed by an abbreviation of the trait name and the chromosome number A final letter was used to accommodate situations where more than one QTL affecting a trait were identified on the same chromosome For the best multiple QTL model a maximum of seven QTLs is allowed in Mapmaker/QTL program If more than seven QTLs were detected for one trait the QTLs which can explain the highest values of phenotypic variation were selected for the regression model

Results

Phenotypic Performance

The mean range values heritability estimates and distributions for three traits viz control root length (CRL) stress root length (SRL) root length ratio (RR) for the DH population and their parents are summarized in table 5 and figure 3 The root of CT9993 and IR62266 showed differential response to aluminum stress CT9993 has a higher SRL and RR indicating its tolerance The range of progenies mean appreciably exceeded their parents for three traits suggesting transgressive variation among genotypes The frequency distribution of CRL SRL and RR of the population was normal according to Shapiro Wilk test The broad sense heritability estimates were 86 92 and 88% respectively for CRL SRL and RR High h^2 values suggest the possibility of exploiting the genetic variation in a breeding selection program

A total of 20 putative QTLs with the LOD threshold of 2 8 were identified for all three traits The putative QTLs lying in their respective chromosome number LOD score percentage of variance explained and allelic effect were presented in Table 6 The number of QTLs identified for individual trait ranged from 3 (for CRL) to 10 (for RR) with the phenotypic variation varying from 9 2 to 28 7% The locations of these putative QTLs for the traits were shown in figure 4 For CRL three QTLs viz *QCrl2a QCrl7a and QCrl8a* were identified with phenotypic variance ranging from 12 0 to 14 8% These three QTLs together explained 34 5% of the phenotypic variation Favorable alleles for *QCrl2a QCrl7a* came from CT9993 (longer root length) but for *QCrl8a* the favorable allele was contributed by IR62266 Seven QTLs *QSrl1a QSrl6a QSrl7a QSrl8a QSrl9a QSrl10a* and *QSrl12a* for SRL were identified on chromosomes 1 6 7 8 9 10 and 12 respectively with the phenotypic variance ranging from 9 2 to 18 3% CT9993 contributed the favorable alleles (longer root length) for all the seven QTLs These QTLs explained 39 9% of the total phenotypic variation A total of 10 QTLs *QAlRr1a QAlRr1b QAlRr2a QAlRr3a QAlRr4a QAlRr7a QAlRr8a QAlRr9a QAlRr10a* and *QAlRr12a* were identified for RR The range of phenotypic variation of the individual QTLs explained was from 10 3 to 28 7% Two QTLs with the largest effect *QAlRr1a* and *QAlRr8a* individually explained 24 1 % and 28 7 % of the phenotypic variation respectively CT9993 contributed favorable alleles (less impaired by stress) for 9 QTLs viz *QAlRr1a QAlRr1b QAlRr3a QAlRr4a QAlRr7a QAlRr8a QAlRr9a QAlRr10a QAlRr12a* and IR62266 contributed favorable allele for only one QTL *QAlRr2a* possibly explaining some of the transgressive variation The best multiple QTL model containing the 7 QTLs with the highest phenotypic variation explained 57 3% of the total phenotypic variance

Comparison of QTLs for Al tolerance Across Rice Genetic Backgrounds

Root length ratio (RR) is the most directly related parameter to Al tolerance in rice and other crops To determine if there are any common QTLs for RR across rice genetic backgrounds results from this study were compared with other reports available in the literature Of 10 QTLs for RR only 2 QTLs were found to be consistent with the QTLs identified in other populations The QTL *QAlRr1a* (R^2=0 241) on chromosome 1 one of the biggest QTLs for RR, was common to QTLs for Al tolerance found in IR1552 x Azucena (Wu et al 2000) and OM269 x Chiembau (Nguyen et al 2001) which also had the largest effect on phenotypic variation (Fig 5) Another genomic region on chromosome 9 harbored *QalRr9a* in this population was found to be common with the minor QTL (R^2=0 09) detected in IR1552 x Azucena population (Wu et al 2000) However the biggest QTL on chromosome 8 (R^2=0 287) was not common with any QTL in IR1552 x Azucena and OM269 x Chiembau populations

Comparison of QTLs for Al Tolerance Among Cereals

To determine whether there are any common QTLs between rice and other cereal species regarding Al tolerance these results were also compared with those of wheat (Riede and Anderson 1996 Aniol and Gustafson 1984) rye (Gallego and Benito 1997 Gallego et al 1998 Aniol and Gustafson 1984) maize (Sibov et al 1999) and barley (Tang et al 2000) using comparative maps (Ahn et al 1993 Ahn and Tanksley 1993 Gale and Devos 1998) and comparative RFLP probe set The analysis did not detect any synteny between the major QTLs controlling Al tolerance in rice population CT9993 x IR62266 and other cereals However a minor QTL (R^2=0 09) on chromosome 3 in Azucena x IR1552 population (Wu et al 2000) was found to be syntenic with the genomic region carrying major Al tolerance gene on group 4 of the Triticeae

Discussion

The genomic region flanked by CDO345 and ME1014 on chromosome 1 appears to harbor the most important QTL associated with Al tolerance in rice By comparing QTLs for Al tolerance among different genetic backgrounds in rice we found the QTL located on chromosome 1 *QAlRr1a* overlapped with the major QTL detected in rice by Wu et al (2000) and Nguyen et al (2001) These results suggested that this genomic region on chromosome 1 contains a major QTL controlling Al tolerance in rice Four QTLs with relatively large effect viz *QalRr8a* (R^2=0 287) on chromosome 8 *QalRr4a* (R^2=0 201) on chromosome 4 *QalRr12a* (R^2=0 197) on chromosome 12 and *QalRr1b* (R^2=0 185) on chromosome 1 were apparently unique in the CT9993 x IR62266 mapping population CT9993 was selected in acid soil conditions and its pedigree consisted of varieties/cultivars being highly tolerant to Al toxicity and low pH such as Moroberekan IRAT216 IRAT13 IRAT120 and IRAT 121 These QTL may explain the high capacity of Al tolerance in CT9993

It has been reported that there is a conserved genomic region on the long arm of homoeologous chromosome 4 for Al tolerance among wheat (*Alt*$_{BH}$) rye (*Alt3*) and barley (*Alp*) The gene controlling Al tolerance of these cereal crops was linked to marker BCD1230 It was suggested that the *Alt*$_{BH}$ *Alt3* and *Alp* genes are orthologous loci because of high level of synteny among chromosome arms 4DL 4RL and 4HL and they may share a common function (Miftahudin et al 2001) One of the Al tolerance mechanisms in the Triticeae is Al exclusion (Delhaize and Ryan 1995 Kochian 1995 Kochian and Jones 1997) This mechanism is mediated by Al activated release of organic acids such as malate or citrate which chelate Al^{3+} in the rhizosphere and prevent its entry into the root apex This physiological evidence is strongly supported by the orthologous loci controlling Al tolerance in the Triticeae species The homoeologous chromosome 4 of the Triticeae corresponds to chromosome 3 in rice (Gale and Devos 1998) However the largest QTLs controlling Al in cultivated rice were located on chromosomes 1 and 8 We hypothesize that the mechanism of Al tolerance in rice might be different from that of the Triticeae species at least in organic acid excretion mechanism Further investigation into the

physiological mechanisms and genes controlling Al tolerance in rice will be beneficial to understanding the evolutionary genetics and diversity of Al tolerance in rice and other grass species

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- **Evaluation and Selection of Inter Specific Populations Via Conventional Breeding Methods**

Introduction

Genetic variability is an essential requirement to make progress in plant improvement programs Traditionally plant breeders make use of diverse genetic resources to come up with improved varieties Most of the time rice breeding programs are under high pressure to deliver superior varieties to meet demands coming from diverse users Crosses using good and well known progenitors have better probabilities of producing the kind of breeding populations from where superior genotypes could be selected Unfortunately not very many good donors are available

and the continued use of them lead to reshuffling of genes reducing at the same time the genetic variability

It has been estimated that around 25% of the total genetic variation available in rice are actually used by rice improvement programs The use of un improved gene pools like wild rice species represents a difficult task to conventional breeding programs however this kind of research is more appropriate to programs more strategic in its scope like the CIAT Rice Project

Therefore activities described in this section deal with the introgression of alleles from wild rice species into the Latin America gene pool using conventional breeding methods The main objective is to develop potential parents to be used by national rice programs

A backcross scheme to diverse improved breeding lines or varieties is used Starting in the F_2 generation single plant selections are made for further evaluation in pedigree rows Around 250 crosses were made and 189 were evaluated under upland conditions in Villavicencio Many populations (119) were discarded because of high sterility and/or poor plant type low yield potential and susceptibility to major diseases A total of 476 plant selections for evaluation as F_3 lines were made

Table 7 presents a summary of inter specific populations evaluated in CIAT Palmira and Villavicencio Advanced lines with good plant type early vigor and excellent grain type were selected from the cross Oryzica 3/*O rufipogon* It was shown (Rice Program Annual Report 2000) that both Oryzica 3 and *O rufipogon* had a good level of resistance to *Rhizoctonia* sp In collaboration with Rice Pathology and FEDEARROZ evaluations are underway under greenhouse and field conditions to test these lines for resistance to Rhizoctonia

Advanced lines (F_6 F_7) with good plant type and yield potential and tolerance to some diseases were selected in the cross Bg90 2/*O rufipogon* Some of these lines have good grain quality and 25 were selected for yield tests in diverse environments through collaboration with national rice programs including Colombia Argentina Venezuela Costa Rica and Honduras

Even after 2 3 backcrosses high sterility has been observed when *O glaberrima* was a parent Despite discard most of the lines F_4 F_5 progenies with high fertility early plant vigor good tillering strong stems and good grain type have been selected Several accessions of *O glaberrima* possess high levels of resistance to the rice stripe virus disease (see section in this report) consequently selected lines will continue to be screened for resistance to rice stripe virus

Crosses between *O barthii* and improved cultivars have resulted in progenies with low yield potential poor plant type and high sterility Nevertheless 2 3 backcross to Lemont have

produced advanced lines with excellent grain type cooking and milling quality early maturity and tolerance to major diseases These lines are susceptible to hoja blanca virus Some lines have better yield potential than Lemont and were included in the VIOAL nurseries for distribution to national rice programs

Two populations (Caiapo/*O glaberrima* and Progresso/*O barthii*) were evaluated under upland savanna conditions in Villavicencio in collaboration with the CIRAD CIAT program Lines from the Caiapo/ *O glaberrima* cross showed good adaptation to acid soils Also doubled haploid lines have been obtained and these are being used to identify and characterize alleles derived from *O glaberrima* that are associated with traits of agronomic importance

In addition 2704 F_4 lines from a collaborative project between CIAT and Peru were evaluated for resistance to rice hoja blanca virus in Palmira and tolerance to major diseases in Santa Rosa, Villavicencio In spite of the high rice blast disease pressure 1097 single plant selections were made for further evaluation as F_5 pedigree rows

Influence of Wild Rice Species on the Grain Quality and Nutritional Value

- **Influence of Wild Rice on the Eating and Cooking Quality of Inter Specific Progenies**

Introduction

The impact of modern agriculture in improving the life and well being of billion of people around the world is impressive The prospect of mass starvation was avoided due to increased food production through the Green Revolution s push for food security However little thought was given to nutritional value and human health and almost none to the concentration of iron and other micronutrients in the new cereal varieties bred Research at IRRI has shown genetic variation for iron and zinc concentration in brown rice Improved cultivars contain about 12 mg of iron and 25 mg of zinc per kilogram while some traditional cultivars have doubled these amounts Results from WARDA (1998) indicates that inter specific crosses with *O glaberrima* gave rise to progenies with higher protein content good eating quality and high nutritive values

CIAT is looking at wild rice species as potential sources of new alleles associated with traits of agronomic importance Although emphasis was given to alleles associated with grain yield and its components we demonstrate that the nutritional and grain quality aspects can also be improved through interspecific crosses as well as within *O sativa*

Materials and Methods

Seed of the advanced lines CT14938 30 5 M 3 and CT14938 36 1 M 1 derived from the cross Lemont/*O barthii* was harvested dried and milled Samples were taken to the quality lab for evaluation Remnant seed was bulked up and milled and 2 kg samples were given to 64 people for cooking and eating evaluations People were advised to cook the rice sample the same way they used at home and to compare its behavior with that of the rice they usually buy and eat

Results

Data from the quality lab showed that both lines had long and slender translucent grains (0 2 white center) with amylose content around 26 29 % and excellent milling recovery (60% head rice) Data from the cooking/eating tests are presented in Figures 6 and 7 Forty seven (75%) people reported that the rice sample was dry and fluffy after cooking whilst 96% of people said that the grain appearance was good before cooking Only 4% found the rice sample to be sticky It is important to keep in mind that the ratio rice/water used by people was different (it ranged from 1/2 2/3 3/4 to 1/1)

Figure 7 shows that 34% of people detected some kind of aroma after cooking and a different taste compared to the rice they usually consume Of the people surveyed 51% reported that the sample given produced more cooked rice than the one they usually consume and 41% said that they were willing to pay a little bit more for type of rice (data not shown)

Results suggest that *O barthii* did not affect in a negative way either the eating or cooking quality of rice On the contrary some people preferred the special features in the quality of the rice derived from an inter specific cross Data also confirm differences in people s preference in terms of grain quality opening up opportunities for the development of special types of rice

- **Influence of Wild Rice on the Nutritional Value**

Materials and Methods

The iron and zinc content was determined for 11 rice cultivars (Table 8) including *O barthii O glaberrima* and *O rufipogon* Brown and milled rice samples were obtained from field plots grown at CIAT and 5 gram each were sent to the lab for chemical analysis The experiment was replicated three times

Results

There were significant differences among cultivars with regard to iron and zinc content in both brown and milled rice as well as in the effect due to milling (Table 8 Figures 8 and 9) As expected brown rice contained higher amount of both iron and zinc than milled rice *O glaberrima* had the highest content of iron with regard to brown rice followed by Fedearroz 50 and Oryzica 1 *O barthii* had the highest content of zinc followed by Fedearroz 50 and three accessions of *O glaberrima* Milling reduced the content of iron by an average of 59% and the content of zinc by 26% There were genotype differences and milling caused a loss of 88% of the iron in *O glaberrima* followed by the breeding line CT 13956 29 29 M (Bg90/*O glaberrima*) IG10 (an acc *O glaberrima*) and the line P1274 6 8 M 1 M It was encouraging to see that CG14 (different acc *O glaberrima*) *O rufipogon* and Oryzica 1 had the highest content of iron after milling suggesting its potential use as parents in a breeding program to increase the content of iron in commercial rice varieties In terms of zinc *O barthii* CG14 IG10 and *O glaberrima* had the highest content after milling

Results suggest that wild rice species can contribute to improve the nutritional quality of commercial rice varieties It is noteworthy to mention that Oryzica 1 and Fedearroz 50 are improved varieties developed in Colombia out of the CIAT/FEDEARROZ breeding programs which possess good levels of iron and zinc even though no breeding effort was made to improve their nutritional value If we make an effort to select for these traits it should not be difficult to develop improve varieties with a better nutritional value

Table 8 Effect of Milling on Grain Iron and Zinc Content of Selected Rice Cultivars

Cultivar	Iron			Zinc		
	Brown Rice	**Milled Rice**	**Milling Effect /**	***Brown Rice***	**Milled Rice**	**Milling Effect /**
Bg90 2	7 2	5 1	**29 1**	17 3	13 9	**19 5**
Barthii	10 4	4 2	**60 1**	27 9	22 0	**21 1**
CG 14	10 8	6 3	**41.3**	24 8	19 7	**20 4**
CT13956 29 M 3 M	10 8	3 0	**72 1**	18 4	11 9	**35.3**
Fedearroz 50	14 0	4 8	**65 9**	25 6	16 7	**35 0**
IG10	12 3	3 7	**70 1**	24 8	18 1	**27 0**
O glaberrima	30 4	3 6	**88 0**	25 0	19 2	**23 3**
Oryzica 1	13 5	6 1	**61 8**	16 5	11 0	**24 2**
Oryzica Llanos 4	13 0	4 9	**54 4**	20 8	15 7	**33.3**
P1274 6 8 m 1 3 M4452	12 3	3 2	**74 2**	13 7	10 5	**23.3**
O rufipogon	10 5	6 2	**41.3**	20 5	15 7	**23 6**
TOTAL	13 2	4 6	**59.3**	21 4	15 9	**26 0**

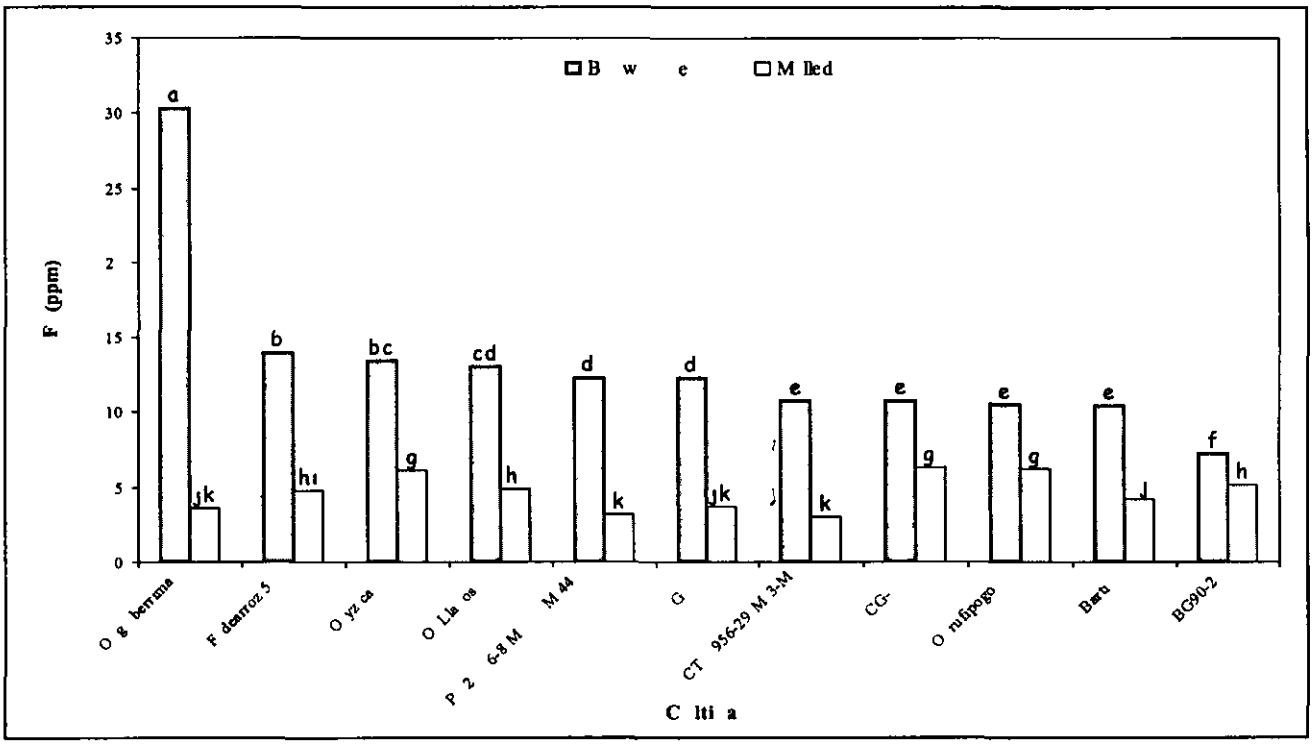

Figure 8 Iron Content in Seed/Grain of Some Selected Rice Cultivars

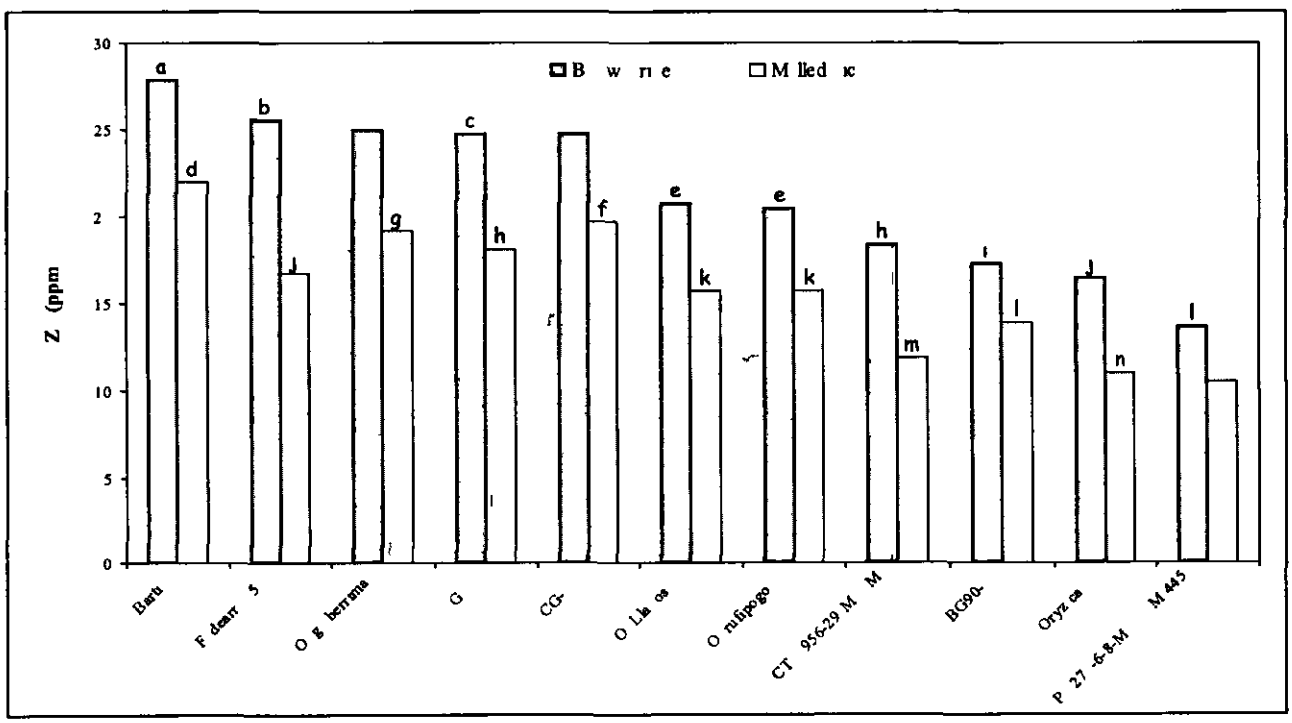

Figure 9 Zinc Content in Seed/Grain of some Selected Rice Cultivars

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- **Population Improvement using Gene Pools and Population with Recessive Male-Sterile Gene**

J Borrero J Carabali C Martinez

Introduction

Recurrent selection is broadly defined as the systematic selection of desirable individuals from a population followed by recombination of the selected individuals to form a new population The process is envisioned as a circle that includes population development evaluations of individuals and selection of superior individuals as parents to form a new population for the next cycle of selection (Fehr 1987) It is a dynamic and continuo process aimed at developing superior genotypes in one or more traits Since 1989 the CIAT rice project has used recurrent selection one of the breeding methods to increase the yield potential as well as resistance to biotic and abiotic production constraints

This activity aims at developing improved populations for irrigated and favored upland conditions having a higher yield potential and good grain quality These are being provided to regional partners to use parental sources and / or lines with specific traits This activity is being done to promote networking among our partners

Materials and Methods

Four base populations (PCT6 PCT7 PCT8 and GPCT9) developed earlier in our rice project were planted and evaluated for yield potential growth duration early vigor plant type and grain quality PCT6 and PCT8 were selected for further improvement in yield potential and grain quality (Annual Report 2000) The main objective is to assess the genetic gain in terms of grain quality after one and two cycles of recombination and selection of the base population In another related study the effect of introducing additional sources of grain quality (Table 9) and

yield potential (Table10) in PCT6 and PCT8 will be determined Experiments are carried out in Palmira under irrigated conditions using a population size of around 1500 plants

Results and Discussion

The first cycle of recombination for grain quality was completed during 2001 Fertile plants (257 and 217 respectively) were harvested in PCT6 and PCT8 After grain quality evaluation seed of selected plants was used to prepare a balanced bulk of seed for each population (PCT6/CG/1/0 and PCT8/CG/1/0) which was then sown for a second cycle of recombination Male sterile plants were identified and marked at flowering time and harvested later on The seed harvested from the male sterile plants was bulked and sown The fertile plants will soon be harvested individually and evaluated for quality The original base populations as well as populations from the first and second recombination cycle will be planted and compared in 2002 to assess the genetic gain in grain quality The new populations (PCT22 PCT23 and PCT24) formed through the introgression of additional sources of grain quality and yield potential underwent the first recombination cycle

Table 9 Sources of Excellent Grain Quality Introgressed into PCT 6 PCT 7, PCT 8 and GPCT 9

Cultivar	Pedigree	Origen	LG	Ac	Fl in	Ht	GW mg	Milling / head	Total	Sh B	Bl	SR	KS m	FSm	BS	EER	Ton/ha EEP
Cypress Inia Tacuari	L 202 / Lemont		L	2	86	38	17 2	66	72	VS	MR	MS	VS	S	R	MR	
Jefferson	Vista/Lebonnet//Rosemont		L	3	79	37	20 2	62	72	S	S	MS	S	MR	R	MR	
L 202	IR456-3 2/unknown semidwarf//l 201	Acc 882															
La Grue	Bonnet73/Nova76//Bonnet73/3/ Newrex	Acc1407	L	3	85	45	18 3	64	72	S	S	MS	VS	S	R	MS	
Lemont	Lebonnet//CI9881/PI331581	Acc 883	L	1	89	35	18 7	63	73	VS	MR	MS	R	MS	R	MR	
Mars	CI9580/Saturn	Acc1114								MS	MS	MS	R		S	VS	
Newbonnet Wells	Dawn/Bonnet73	Acc 884	L	3	87	45	15 6	66	72	MS	VS	S	VS	VS	R	MR	
Kaybonnet	Katy/Newbonnet		L	5	84	44	15 3	65	72	MS	R	MS	MS	S	S	MS	
Drew	Newbonnet/Katy		L	4	87	46	16 0	65	72	MS	R	MS	MS	VS	S	MR	
Katy	Bonnet73/CI9722//Starbonnet/T etep/3/Lebonnet									MS	R	MS	R	MR	R	S	
Millie	Lebonnet/L 201									MS	MS	MS	MR		R	S	

LG=Grain length (L=long/slender M=Medium)
Ac= Lodging (0=Strongn stems 9=Weak stems)
GW= 1000 grain weight (mg)
R= Resistant MR=Moderately Resistant, MS= Moderately Susceptible S=Susceptible VS=Very Susceptible
ShB= Rhizoctonia Solani
Bl= Pyricularia Oryzae
SR= Pudricion de Tallo (Magnaporthe Salvinii)
KSm= Carbon (Tilletia barclayana)
Fsm = Falso Carbon (Ustilaginoidea Virens)
BS = Helminthosporium
EER = Espiga Erecta

Table 10 High Yielding Cultivars Introgressed into PCT 6,PCT 7, PCT 8 and GPCT 9

Pedigree	Origen	VG	FL	HT	LSC	BS	GD	BL1	BL2	NBL	HB	HB	HB	CB	LG	AMY	TGEL	Yield Ton/ha
IET13652	India	1	90	72	1	3	5	4	5	3	9	9	9					6 9
C15	Vietnam	1	104	78	3	3	3	2	3	1	9	9	9	3 6	3	32 0	B	6 6
SPR88090 30 1 2 2		1	99	73	3	1	5	3	4	5	9	9	9					7 0
ITA406	IITA	3	114	72	1	1	5	5	7	5	9	9	7					6 5
SPR87036 7 1 1 2		1	102	89	3	3	7	3	3	5	9	9	7					6 8
IR65469 161 2 2 3 2 2	IRRI	3	112	69	3	3	7	7	7	5	9	9	7					7 5
IR72102 3 115 1 3 2	IRRI	3	90	68	1	1	5	5	5	7	9	9	9					6 7
IR70183 74 1 1 1	IRRI	1	94	88	3	1	5	2	1	3	5	9	9					6 7
PSB RC 68	IRRI	1	114	10	3	1	5	1	1	5	9	5	5					7 2
PSB RC 70	IRRI	1	112	89	3	1	5	2	2	5	7	9	9	0 4	3	25 9	B	6 6
IR68835 93 2 B 1 1 1	IRRI	1	96	125	3	1	5	4	5	7	9	9	9					7 7
IR70177 76 2 1 1	IRRI	1	94	90	3	1	7	3	4	1	9	9	9					7 0
IR69504 48 SRN 2 UBN 1 2	IRRI	1	98	86	3	3	3	1	2	3	9	9	9	2 6	3	27 9	B	8 3
IR70169 3 2 1 2	IRRI	3	103	79	3	1	3	3	4	7	9	9	9					7 6

LG=Grain lenght (L=long/slender M= Medium)
Ac= Lodging (0=Strong stems 9=Weak stems)
VG=early vigor FL=Flowering HT=Plant height CB=White center Amy=Amylose /
Disease reaction Escale 0 9 0 3=Resitant 9=Highly susceptible
LSC=Leaf scald BS=Brown spot GD=Grain descoloration HB=Hoja blanca virus disease
Bl= Pyricularia Oryzae
BS = Helminthosporium

OUTPUT 1 ENHANCING GENE POOLS

1 D Introgression of New Plant Type Genes into LAC s Gene Pools

J Borrero C Martinez C Bruzzone

Introduction

Ideotype breeding aimed at modifying the plant architecture is a time tested strategy to achieve increases in yield potential To increase the yield potential of rice further a new plant type (NTP) was conceptualized by IRRI scientists in 1988 Further modifications in plant architecture were proposed with the following characteristics low tillering capacity no unproductive tillers 200 to 250 grains per panicle very sturdy stems dark green thick and erect leaves and vigorous / deep root system Numerous breeding lines with desired ideotype were introduced to CIAT from IRRI and evaluated in Palmira and Santa Rosa for adaptation yield potential grain quality and tolerance to main biotic and abiotic stresses Several promising lines were identified and used as parents in our breeding program This activity is aimed at incorporating important agronomic traits exhibited by the NPT material into gene pools for use in Latin America and the Caribbean

Materials and Methods

Twenty eight F_1 populations were obtained in 2001 from crosses having at least one dosage of a NPT parent and two dosages of a locally adapted cultivar In some cases wild rice species were also used as parents Another 307 F_2 populations derived from crosses made in 2000 were evaluated under rain fed conditions in Santa Rosa as well as 391 F_4 pedigree rows

Results

Good genetic variability was observed in the F_2 populations especially in terms of plant type tillering panicle size plant height and leaf senesce Nearly 60% of the populations were discarded because of susceptibility to diseases Around 463 single plant selections were made for further evaluation as F_3 pedigree rows in 2002 Out of the 391 F_4 lines evaluated 177 plant selections were made for evaluation in 2002

Discussion

Rice of the NPT needs extensive adaptation to LAC if it is to be successful These plants have many useful traits that could enhance rice The use of molecular markers could speed up these incorporation of these traits into breeding populations and ultimately commercial varieties Both the breeding of the NPT and the development of molecular markers for selected traits will become part of other activities next year

OUTPUT 1 ENHANCING GENE POOLS

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

Abstract

The current report illustrates the use of doubled haploids in the different projects involving rice breeding at CIAT Doubled haploids lines were generated for the recurrent selection and pedigree selection methods used for CIAT to broaden the genetic base of rice in Latin American Double haploids can be generated from single double or triple crosses within rice or with wild species as well as from fertile or highly sterile plants CIAT collaborates to produce double haploids for the FLAR breeding program Once doubled haploids are produced these plants are subject to the evaluation process jointly with the other breeding population Although only a faction of a percentage of crosses were processed using anther culture it is worthwhile to point out that in the last year about 20% of the lines sent for evaluation in Latin America or selected by the countries were anther culture derived materials

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 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 focuses on developing doubled haploid lines for the various breeding efforts 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 back-crossed 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 sub tropical and cold tolerant breeding pools for the Southern cone and produced somaclonal lines for Tropical Latin America From September 2000 to September 2001 the ACL generated a total of 7 542 plants

Materials and Methods

From October 2000 to September 2001 a total of 168 000 anthers were cultured Plants were grown in the field and the panicles were harvested After a cold treatment the anthers were 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

Results and Discussion

- **Use of Anther Culture to Fix Enhanced Traits in Hybrid Back Cross Populations of Rice and Wild Species**

A Mora (IP4) G Delgado (IP4) T Agrono (IP4) C Ordonez (IP4) E Bolaños (IP4) A Rios (IP4) J Trigreros (IP4) J Carabali (IP4) C Martinez (IP4 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 in order to by passed the sterility bottleneck and generate advanced lines for the back cross program This year 911 plants were generated from advanced back crossed populations of *O sativa/O barthii* and *O sativa/O glaberrima* A total of 354 R_3 lines from the cross Lemont/*O barthii* were generated in 2000 and were evaluated in Santa Rosa Experimental Station the first semester 2001 From these 17 lines were selected based on disease resistance Of the 99 BC_2F_2 and BC_3F_1 lines from the cross Caiapo *O glaberrima* processed through anther cultured last year a total of 312 DH lines were selected for QTL yield trials and disease resistance These will be evaluated at Louisiana State University by Dr Gabriel Aluko and at Santa Rosa Experimental Station for disease resistance

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

A Mora (IP4) G Delgado (IP4) T Agrono (IP4) C Ordonez (IP4) E Bolanos (IP4) A Rios (IP4) J Trigreros (IP4) J Carbali (IP4) C Martinez (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 derived from the new plant type are being evaluated for RHBV resistance as well as

some components of yield including tiller capacity days to flowering panicle length fertility and grain size at CIAT experimental station The derived R_3 population with be tested for rice blast resistance at the Santa Rosa station Advanced R_4 will be available to national programs Last year it was reported a total of 318 R_2 crosses that included the new plant type were selected 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 and 53 R_3 lines selected by Francisco Andrade (Ecuadorian breeder) at CIAT which were sent to Ecuador in 2001

- **Use of Anther Culture to Fix Cold Tolerance in Recurrent Selection Populations**

A Mora (IP4) G Delgado (IP4) T Agrono (IP4) C Ordonez (IP4) E Bolanos (IP4) A Rios (IP4) J Trigreros (IP4) J Borrero (IP4) Y Ospina (IP4) M Chatel (IP4) Z Lentini (IP4)

Last year a total of 325 R_2 plants were generated from two different recurrent selection populations carrying male fertility restorer gene along with cold tolerance These lines were sent to Chile to be planted in November 2000 for final field evaluations Cold tolerant plants were then sent to France and lines showed early flowering high yield and good quality

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

Last year a total of 325 R_2 plants were generated from two different recurrent selection populations carrying male fertility restorer gene along with cold tolerance These lines were sent to Chile to be planted in November 2000 for field evaluations Cold tolerant plants were then sent to France and lines showed early flowering high yield and good quality

A Mora (IP4) G Delgado (IP4) T Agrono (IP4) C Ordoñez (IP4) E Bolanos (IP4) A Rios (IP4) J Trigreros (IP4) L E Berrio (FLAR) J Gibbons (FLAR) Z Lentini (IP4)

Last year 21 R_3 lines generated from crosses processed in 1999 were selected for disease resistance at Santa Rosa and for plant type and grain quality at CIAT headquarters VIOFLAR 2000 for the Southern Cone included total of 175 lines of which 12% were AC derived lines) Four R_3 lines (19%) out of the 21 R_3 lines were selected for yield potential by Argentina Brazil and Uruguay In 2001 94 R_2 plants derived from 190 triple crosses designed for Southern Brazil were selected both at CIAT experimental station for plant and grain type and at Santa Rosa Experimental Station for disease resistance These 94 R_2 were sent to Brazil with other selected 25 R_3 lines in 2001 and will be evaluated in Brazil next season

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

A Mora (IP4) G Delgado (IP4) T Agrono (IP4) C Ordonez (IP4) Edgar Torres (Fonaiap Venezuela) J Holguin (Fedearroz Colombia) J Gibbons (FLAR[1]) Z Lentini (IP4)

Responding to a request from FLAR research group at CIAT headquarters Colombia and Venezuela as members of FLAR the ACL had generated somaclone lines derived from immature inflorescence using selected varieties The goal of this activity is to induce variation for improving grain quality traits RHBV resistance tolerance to *Tagosodes* mechanical damage and lodging tolerance Of the 4 440 somaclone plants generated for Fedearroz Colombia none of them showed increased amylose content which was the main objective of this activity In the case of Fundarroz Venezuela, 3 178 somaclones were generated last year The S1 seeds (first self of the original somaclone S0) was harvested and S1 plants were evaluated for grain quality and lodging tolerance and S2 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 Eventhough some promising somaclone lines had been generated from this activity CIAT had discouraged FLAR to use this approach for generating variants due to its low efficacy since most variants are usually of epigenetic nature

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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**

F Correa Didier Tharreau (CIRAD) F Escobar G Prado G Arcapa

Abstract

This study was initiated to understand the evolutionary response of the genetic and virulence structure of blast populations when subject to the selection pressure of rice cultivars with different levels of resistance Near isogenic lines with different blast resistance genes and combinations were developed and used in studies on their effect on blast development Certain avirulence genes were associated with fitness in the 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 critical avirulence genes associated with pathogenic fitness

Introduction

The current experiments aim at following the changes that occur in blast population genetic and pathogenic (virulence and aggressiveness) when subjected to selection pressure on varieties with either complete or partial resistance as well as susceptibility to the pathogen In the field the resistance level of the varieties was evaluated by mean of disease ratings Relations between blast population changes and evolution of disease level will be examined for a period of three years

Materials and Methods

The experiment was conducted at the Santa Rosa station Fedearroz 50 and Oryzica Llanos 5 were the varieties with complete resistance Ceysvoni Oryzica Llanos 4 IR 36 Iniap 11 (IR 64) and Oryzica 2 have partial resistance Oryzica 1 Oryzica Caribe 8 Cica 8 and Oryzica 3 were the susceptible varieties Each variety was sown every 15 days for a total of 6 planting dates in plots 5x5 meters 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 diseased leaves 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 on the blast samples using PCR DNA fingerprinting and greenhouse inoculations

Results

DNA samples from more than 100 isolates of *Pyricularia grisea* 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 analyzed so far were clustered in three major groups corresponding to the known Colombian genetic lineages SRL-6B SRL 6 and SRL-4 Differences in genetic structure over all years and planting dates are being analyzed in replicated experiments in order to be associated to evolutionary processes and response to the selection pressure of the rice cultivars with different resistance levels Complete results will be presented in next year annual report after analyzing all the information together The spectrum of virulence of isolates 1 to 4 represented some of the new genotypes that were analyzed in greenhouse studies (Table 1) None of the new isolates defeated all known plant resistance genes The new isolates differed in their spectrum of virulence in comparisons made between themselves or with the standard isolates representing the lineages SRL 2 to SRL 6 (Table 1) The new isolates differed in the number and kind of avirulence genes with cases where only a few of the known resistance genes were effective against the isolates (Table 1) These results suggest changes in virulence patterns where these isolates lost some of their avirulence genes and were able to overcome more resistance genes than standard isolates (Table 1) The only resistance genes effective against the new isolates are Pi 1 Pi k^h Pi 11 Pi ta^2 Pi z and Pi b None of these genes were effective against all the isolates however appropriate combinations of these genes should confer resistance against them

The near isogenic line C 101 LAC has both the Pi 1 and Pi 11 resistant genes and these were separated into two near isogenic lines The new near isogenic line CT 13432 6 carried the Pi 11 gene and exhibited resistance to lineages 2 and 6 while the C104LAC line carried the Pi 1 gene and was resistant to lineages 2 4 and 6 In addition the C101 A51 line that carried the Pi 2 gene conferred resistance to lineage 5 (Table 1) In separate field and greenhouse experiments (data not shown) the combination of these three genes in a near isogenic line conferred resistance to all the genetic known lineages of the blast pathogen in Colombia at both the leaf and panicle stages These three genes are being incorporated via backcrossing and marker assisted selection to several Latin American rice varieties as will be shown later in this report

We have detected for the first time a fully compatible isolate with the rice variety Oryzica Llanos 5 in greenhouse inoculations (isolate 1 Table 2) Although the variety Oryzica Llanos 5 still exhibits a resistant reaction under field conditions this new isolate caused severe infection in the artificial inoculations in the greenhouse We are in the process of analyzing the blast populations and the changes that have occurred from 1999 through 2001 at Santa Rosa Among the expected outcomes will be the frequency of novel isolates and their importance Most Colombian commercial rice cultivars were susceptible to the isolate 1 which is compatible with the variety Oryzica Llanos 5 The exceptions were the cultivars Oryzica 2 Cica 8 and Fedearroz 50 (Table

2) Isolate 1 seems to be a member of lineage SRL 4 which has apparently lost at least two or three avirulence genes (Tables 1 and 2) Isolate 3 was found to be partially compatible with the cultivar Fedearroz 50 (Table 2) Isolate 3 exhibited a partially compatible reaction with both Fedearroz 50 and Oryzica Llanos 5 and was tentatively grouped in the lineage SRL–4 Additional studies are being carried out to confirm these observations Our results suggest that lineage SRL 4 is evolving and has lost at least the avirulence genes for Pi ta^2 Pi k and Pi b (Table 1) thereby gaining virulence with the cultivars Oryzica Llanos 5 and Fedearroz 50

By interpreting the reaction to rice blast isolates we propose that the cultivars Oryzica Llanos 5 and Fedearroz 50 have at least 8 similar major resistance genes (Table 3) Oryzica 2 is estimated to contain at least 7 major resistance genes These three cultivars seem to have in common the resistance genes Pi 2 Pi ta^2 Pi sh Pi k and Pi b Oryzica 2 differs from O Llanos 5 and Fedearroz 50 in at least 5 resistance genes The cultivars O Llanos 5 and Fedearroz 50 carry the resistance genes Pi 11 Pi z and Pi z^t while the cultivar Oryzica 2 possesses the genes Pi 1 and Pi k^h (Table 2) Cica 8 is expected to have at least 4 major resistance genes

We have demonstrated in previous years that the combination of the resistance genes Pi 1 Pi 2 and Pi 11 confers resistance to all the known pathogen diversity present in Colombia (see previous annual reports) This has been observed despite the fact that compatible isolates with each one or combination of two of the three resistance genes have been detected in the pathogen population (Table 1) We think that losing the corresponding three avirulence genes has fitness costs to the pathogen If this hypothesis is correct then the combination of the resistance genes Pi 1 Pi 2 and Pi 11 should confer a more durable resistance than the other resistance gene combinations

None of the Colombian commercial varieties seem to carry the combination of the three resistance genes Pi 1 Pi 2 and Pi 11 (Table 3) No variety appears to carry the combination of Pi 1 and Pi 11 It is interesting to note that the cultivar Oryzica Llanos 5 which has exhibited a durable blast resistance since 1989 only contains Pi 2 and Pi 11 (Table 3) However this cultivar also carries the gene Pi k which seems to be an allele located at the same locus as the gene Pi 1 in chromosome 11 This three gene combination could have been conferring the durable resistance to O Llanos 5

We have always asked ourselves if the most predominant lineage in a pathogen population would be responsible for the breakdown of resistance of new rice cultivars Lineage SRL 6 has always been the most predominant in the Colombian blast pathogen population However breakdown of the resistance of Oryzica Llanos 5 has been caused by lineage SRL 4 If our hypothesis that the combination of the three avirulence genes in the blast pathogen avr Pi 1 avr Pi 2 and avr Pi 11 is associated with the fitness of the pathogen then according to Tables 1 and 3 lineage SRL 4 was in a better position than lineage SRL 6 for changing and breaking down the resistance of the cultivar First lineage SRL 6 has already lost the avirulence genes avr Pi 1 and avr Pi 2 (Table 1) and would have to lose avr Pi 11 since the resistance gene Pi 11 seems to be present in

Oryzica Llanos 5 (Table 3) Losing the three avirulence genes would be deleterious for the pathogen Second lineage SRL-4 has already lost the avirulence genes avr Pi 2 and avr Pi 11 (Table 1) however it is not necessary to lose avr Pi 1 as the gene Pi 1 is not present in the cultivar Oryzica Llanos 5 (Table 3) Therefore changes in lineage SRL 4 would not have a deleterious effect on the pathogen

Why has it taken so long for lineage SRL 4 to change and breakdown the resistance of Oryzica Llanos 5? There could be many explanations including lineage SRL 4 was always in very low frequency and has only increased recently after the release of other rice cultivars highly susceptible to lineage SRL 4 Second Oryzica Llanos 5 has at least 8 major resistance genes (Table 3) Third the most predominant lineages SRL 6 and SRL 4 carry the avirulence genes avr Pi ta^2 and avr Pi k which could also be associated with pathogen fitness The corresponding resistance genes seem to be present in the cultivar Oryzica Llanos 5 (Table 3) Fourth Oryzica Llanos 5 still exhibits a partial resistance in the field which could indicate the presence of minor resistance genes which in combination with the major genes could have been responsible for the durability of the resistance in this cultivar Fifth the detected isolates may never play a role in nature and Oryzica Llanos 5 could continue being resistant in the field

Discusion

Development of durable blast resistance is one our major goals at the CIAT Rice Project as it is cost effective environmentally sound and protects human health and genetic resources We have been studying the blast pathogen interactions with the rice plant for the last ten years in order to understand the changes and evolution that are taking place in the pathogen in order to overcome the genetic resistance deployed in the newly released rice varieties We have found that certain avirulence gene combinations in the pathogen seem to be very critical to pathogenic fitness and this knowledge could be very important to predict the durability of resistance gene combinations and their use in rice breeding for disease resistance

The concept that durable resistance is a reflection of pathogen fitness is well established The inherent quality and durability of a plant resistance gene is a direct function of the amount of fitness penalty imposed on the pathogen to overcome that resistance gene (Leach et al 2001) This means that a mutation from avirulence to virulence can be associated with a fitness penalty and the pathogen can suffer from a reduction in fitness on the host In our studies we have been linking many greenhouse and field studies to determine the role of certain avirulence genes in the fitness (competition reproduction multiplication infection efficiency amount of disease produced etc) of the blast pathogen We are using this information to develop a sound breeding strategy for achieving durable blast resistance We have developed a hypothesis that predicts which combinations of major resistance genes should be durable This hypothesis will be tested using both conventional breeding and by using marker aided selection The molecular markers will also be useful to confirm the biological data and test if our predictions of major resistance gene combinations in the commercial varieties are correct

Future Activities

The three year study on the evolution of the blast pathogen as a result of the selection pressure imposed by rice cultivars with complete and partial resistance as well as susceptibility will be completed during the next year Research to determine the effect of certain avirulence gene mutations on the fitness of the blast pathogen and its relation to natural evolution of avirulence/virulence genes will continue as will the studies to determine the frequency of avirulence/virulence genes in the presence/absence of corresponding resistance genes These studies will be facilitated by the increasing information on the genome of rice and the rice blast fungus The near isogenic lines will be the key to the efforts to determine the effect of a different genetic background on the role of a particular avirulence gene in fitness

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Table 1 Avirulence Genes Detected in *Pyricularia grisea* Isolates Collected in Evolution Studies of the Blast Pathogen

Source	Resistance Gene	Isolate/Genetic Lineage								
		1[1]	2	3	4	5	6	7	8	9
		?*[2]	?	?*	?*	L6	L4	L5	L2	L6
C 104 LAC	P_1 1	R[3]		R	R		R		R	R
C 101 A51	P_1 2							R		
CT 13432 6	P_1 11		R			R			R	R
C 104 PKT	P_1 3								R	
C 101 PKT	P_1-4a								R	
C 105 TTP4 (L23)	P_1 4b								R	
F 124 1	P_1 ta									
F 128 1	P_1 ta^2				R		R			R
F 80 1	P_1 k						R			R
F 98 7	P_1 k^m									
F 129 1	P_1 k^p									R
F 145 2	P_1 b			R						R
Aichi Asahi	P_1 a									R
K 3	P_1 k^h	R		R	R		R		R	R
K 59	P_1 t									
Rico 1	P_1 k^s									
Norin 2	P_1 sh							R		R
Nipponbare	P_1 sh							R		R
Nato	P_1 1									
OU 244	P_1 z		R			R		R	R	R
Toride	P_1 z^t					R		R		R

1 Isolates 1 # 27 2 OY9 17 1 3 F 50 24 1 4 OLL5 304 5 FAN 54 (Lineage SRL 6) 6 OC8 17 1 (Lineage SRL-4) 7 FAN 47 1 (Lineage SRL 5) 8 C9 37 1 (Lineage SRL 2) 9 CEY 19 1 (Lineage SRL 6)

2 ?* = Not determined

3 R = Incompatible or resistant reaction as a result of the interaction between an avirulence gene in the pathogen and a resistant gene in the host plant

Table 2 Blast Reaction of Colombian Commercial Rice Cultivars Inoculated with *Pyricularia grisea* Isolates Representing most Virulence Diversity in Colombia

	Isolate/Genetic Lineage								
	1[1]	2	3	4	5	6	7	8	9
Rice Cultivar	?[2]	?	?*	?	L6	L4	L5	L2	L6
Metica 1	+[3]	+	+	+	+	+			+
Oryzica 1	+	+	+	+	+	+		+/	+
Oryzica 2		+			+				
Oryzica 3	+	+	+		+				+/
Cica 7	+	+	+	+	+	+			+
Cica 8		+			+		+		
Cica 9	+	+	+		+			+	
IR 22	+	+	+		+		+/		+/
Oryzica Llanos 5	+		+/	+/		+/			
Linea 2	+	+	+		+			+	
Oryzica Llanos 4	+	+/	+		+	+/			
Oryzica Caribe 8	+	+/	+	+	+	+			
Oryzica Yacu 9	+	+	+		+				
Fedearroz 50			+/						

1 Isolates 1 #27 2 OY9 17 1 3 F 50 24 1 4 *OLL5* 304 5 FAN 54 6 OC8 17 1 7 FAN 47 1 8 C9 37 1 9 CEY 19 1

2 ?* = not determined

3 + = Susceptible or compatible reaction +/ = a low level of disease reaction

Table 3 Possible Blast Resistance Genes present in Colombian Commercial Rice Cultivars Inferred from Inoculations with Isolates Carrying Corresponding Avirulence Genes

	Resistance Gene									
Rice Cultivar	**Pi 1**	**Pi 2**	**Pi 11**	**Pi z**	**Pi z[t]**	**Pi ta[2]**	**Pi sh**	**Pi k[b]**	**Pi k**	**Pi b**
Metica 1										
Oryzica 1										
Oryzica 2	X[1]	X				X	X	X	X	X
Oryzica 3						X			X	
Cica 7										
Cica 8	X					X			X	X
Cica 9		X				X				
IR 22						X	X		X	
Oryzica Llanos 5		X	X	X	X	X	X		X	X
Linea 2		X								
Oryzica Llanos 4		X					X		X	
Oryzica Caribe 8		X					X		X	
Oryzica Yacu 9		X								
Fedearroz 50		X	X	X	X	X	X		X	X

1 X = Presence of Resistance Gene

- **Selection of Rice Blast Resistance Sources to Different Blast Genetic Lineages under Greenhouse and Field Conditions Development of a Blast Nursery with Potential Sources of Resistance**

F Correa, G Prado G Arıcapa, C Martınez

Abstract

The frequency of blast resistant plants ın F_2 populations ıs highly dependent on the blast reactıon and stabılity of the parents used for the development of these populatıons We have ınıtıated the development of a nursery wıth potentıal sources of durable blast resıstance Advanced rıce lınes are beıng evaluated for at least seven seasons under hıgh dısease pressure and only hıghly and durable resıstant lınes wıll be ıncorporated ınto the nursery Thıs nursery wıll be used as the source of parents for breedıng programs ın Latın Amerıca New blast resıstance genes are beıng ıdentıfied ın the wıld rıce relatıve *O rufipogum*

Introduction

The frequency of blast resistant plants observed ın F_2 populatıons ın the field ıs hıghly dependent on the blast reactıon and stabılity of thıs reactıon of the parents used for the development of these populatıons An ıncrease ın the number of susceptıble F_2 plants was found durıng year 2000 as compared to previous years Thıs was related to the low stabılity of the blast resistance of the parents used ın the breedıng programs We have ınıtıated the blast evaluatıon over tıme ın the field and greenhouse of several hundred advanced as well as segregatıng lınes exhıbıtıng desıred agronomıc traıts to ıdentıfy sources of blast resistance These populatıons ınclude lınes derıved from ınterspecıfic crosses that were made ın order to ıdentıfy new resistance genes We are developıng a nursery of potentıal sources of blast resistance to be used as parents and wıll dıstrıbute them to partners ın Latın Amerıca for testıng and use ın theır breedıng programs

Materıals and Methods

Rıce lınes were evaluated for theır reactıon to blast The best resistant lınes were selected from a varıety of dıfferent breedıng materıals ıncludıng advanced lınes lınes from the germplasm bank recurrent selectıon breedıng lınes that were focused on blast resistance and lınes derıved from ınterspecıfic crosses The selected lınes were planted at the Santa Rosa experıment statıon usıng spreader rows as source of ınoculum The spreader rows whıch were planted three weeks before the lınes beıng evaluated consisted of a mıxture of cultıvars susceptıble to all known genetıc lıneages of the blast pathogen present ın Colombıa Evaluatıng lınes were planted perpendıcular to the spreader rows ın two rows per lıne wıth two replıcatıons The lınes were planted wıth a seed densıty of 2 3 grams per meter ın 2 meters rows A total of 180 kg of N/ha was applıed ın

split applications at 15 30 50 and 70 days after planting to favor blast development Leaf and panicle blast was evaluated on a scale of 0 9 at 30 40 and 50 days after planting and 25 and 32 days after flowering respectively Those lines with a score of 0 3 for both leaf and panicle blast were selected and included in a blast nursery for future evaluations under greenhouse and field conditions for the selection of potential sources of stable resistance to blast after several seasons of evaluations The selected lines will be tested in the greenhouse with blast isolates representing most of the virulence and genetic diversity of the pathogen found recently in our studies Selected isolates will include those recovered from highly resistant lines exhibiting blast lesions in low frequency to detect possible mutations of avirulence genes in the pathogen

Results

A total of 100 advanced rice lines with resistant leaf and panicle blast scores 0 3 were selected in 2000 and tested under field conditions at Santa Rosa in 2001 Table 4 includes the twenty two most resistant lines selected from this group and these were included in the blast nursery of potential sources of resistance These lines will continue being evaluated under field conditions as well as greenhouse inoculations to confirm their resistance and stability before their use as parents A recurrent selection program initiated several years ago for blast resistance and including 30 parents of highly diverse genetic background and blast resistance yielded many blast resistant lines Ten advanced lines from this program have exhibited a stable leaf and panicle blast resistance for seven years including evaluations in two replicated trials during 2001(Table 5) These lines exhibited a highly resistant panicle reaction which is highly desired in a resistance source being used as a parent These lines have been included in the nursery of sources of resistance to blast More than 400 advanced lines from the FLAR s germplasm bank were characterized for their blast resistance in 2001 and lines with leaf and panicle blast scores 0 2 were selected for future characterization of their resistance in replicated trials (Table 6) It is interesting to note that the best resistant lines identified correspond to new lines selected in FLAR crosses (FL) while old advanced lines included in the bank tended to be more susceptible The selected lines will be tested again in 2002 and the most resistant ones will be included in the nursery of sources of resistance

Greenhouse as well as field studies conducted in the past few years using Colombian blast populations have indicated the presence of virulence factors to most known blast resistance genes in rice These results have suggested the need to identify suitable gene combinations for blast resistance as well as the need to identify additional resistance genes from either the cultivated species *O sativa* or related species The search for additional blast resistance genes was initiated using *O rufipogum* and *O glaberrima* both of which exhibit resistance to blast The wild species *O barthii* exhibited a highly susceptible reaction to blast Table 7 shows the percentage of blast resistant lines in 204 lines from the cross between the commercial rice cultivar Bg90 2/*O rufipogum* evaluated under greenhouse and field conditions Both parents exhibit a susceptible field reaction (Table 7) which has been demonstrated in greenhouse inoculations to the genetic lineage SRL-5 The two parents are resistant to the lineages SRL 1 2 3 4 and 6 The two parents exhibited a differential reaction to two isolates of the blast pathogen

within lineage SRL 5 (Table 7) Inoculations with isolate 1 to which Bg90 2 was resistant/intermediate and *O rufipogum* was susceptible only 4 9% of the progeny were resistant This result suggests the presence of a very few weak genes in the resistant parent Bg90 2 It is possible that the intermediate reaction observed in Bg90 2 to isolate 1 indicates the potential for a breakdown of the resistance Inoculations with isolate 2 to which *O rufipogum* is resistant and Bg90 2 susceptible 84 3% of the progeny were resistant This result suggests the presence of potentially useful resistance genes in the *O rugipogum* Field evaluations of the same lines (Table 7) yielded more resistant lines at the leaf stage (59 8%) than at the panicle stage (8 3%) Since both parents were susceptible to leaf and neck blast these results suggests a probably low effect of the resistance genes present in the wild species for panicle blast control to lineage SRL 5 It is possible that most resistance genes present in *O rufipogum* are minor genes which have not enough resistance to control panicle blast More research is however needed to confirm this hypothesis Those lines with a panicle resistant reaction can possibly carry a combination of minor genes present in the wild species that could be useful to control neck blast One line derived from this cross (CT 13946 1 1 M M 2) exhibited a resistant reaction to both isolates in greenhouse and field evaluations This line will be further evaluated before being included in the nursery of potential donors of resistance

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 target 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 generations (Correa and Zeigler 1995) It is possible that only after several generations of exposure that the most effective resistance genes can be identified These genes at the same time should correspond to those avirulence genes more associated with fitness in the pathogen and which have 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 of stable blast resistance sources that should be used in breeding programs that aim to developing varieties with durable blast resistance This nursery is being formed on the basis of advanced breeding lines exhibiting stable blast resistance This nursery will be subject to high blast pressure every year to assure 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 stable resistance to blast The search for new blast resistance genes will continue and *O rufipogum* and *O glaberrima* seem to carry novel genes These activities are part of the development of a nursery with sources of stable blast resistance

Table 4 Rice Lines Exhibiting a Highly Resistant Leaf and Panicle Blast Reaction (1 3) to Lineages SRL 1 to SRL 6 in the Greenhouse and the Field at Santa Rosa during 2000 and 2001 Evaluations

Number	Rice Line
1	CT 8455 1 24 3P 1X
2	CT 8008 3 12 3P 1X
3	CT 8238 6 13 1P 1X
4	CT 9737 1 1P 2 1
5	VSTA/LBNT//RSMT
6	GFMT*2/TQNG
7	CT 11280 2 F_4 12P 5
8	CNAX 5013 13 2 2-4 B
9	CT 11369 1 F_4 17P-4P
10	CNAX 5013 12 13 2 2-4 B
11	CT 13503 M 3 1 M 2 1P
12	CT 13503 M 3 1 M 2-4P
13	FL 00447 27P 3 1P M
14	FL 00470 29P 5 2P M
15	FL 00470 29P 6 2P M
16	FL 00470 29P 7 3P M
17	FL 00585 12P 7 3P M
18	FL 00593-6P 1 3P M
19	FL 00593 6P 5 3P M
20	FL 00593 6P 7 1P M
21	FL 00595 12P 1 1P M
22	FL 00595 25P 9 3P M

Table 5 Rice Lines Developed in a Recurrent Selection Project for Blast Resistance that have Exhibited a Stable Leaf and Panicle Blast Resistance for Seven Years

Pedigree	Leaf[1] Blast 1	Leaf Blast 2	Leaf Blast 3	Neck Blast 1	Neck Blast 2	Leaf Scald	Brown Spot	Grain Discolor
CT 13448 M 8 1 M	1[2]	2	2	1	1	5	3	3
CT 13449 M 8 2 M	1	1	1	1	1	5	3	3
CT 13449 M 6 1 M	3	1	2	1	1	6	5	3
CT 13458 M 3-4 M M	1	1	2	1	1	7	3	3
CT 13462 M 12 2 M M	1	1	1	1	1	5	3	3
CT 13462 M 13 1 M M	1	1	2	1	1	5	3	3
CT 13465 M 6 1 M M	1	1	1	1	1	5	3	3
CT 13501 M 13 1 M	2	3	3	1	1	5	3	5
CT 13503 M 3 1 M	1	1	2	1	1	5	3	3
CT 13503 M 13 1 M	1	1	2	1	1	5	3	3

1 The lines were evaluated for leaf blast 30 40 and 50 days after plant and for neck blast at 25 and 32 days after flowering 2 Rice diseases evaluated on a scale of 1 9 (1= highly resistant 9= highly susceptible)

Table 6 Rice Lines from FLAR Germplasm Bank Exhibiting a Highly Resistant Leaf Neck Blast Reaction (1 2) at Santa Rosa in 2001

Number	Rice Line
1	FL 00470 29 2 3P M
2	FL 00147 8P 6-15P M
3	FL 00447 35P-4 2P M
4	FL 00826 6P 5 1P M
5	FL 00854 22P 2 3P M
6	FL 00854 22P 3 2P M
7	FL 00855 1P 2 2P M
8	FL 00871 1P 5 1P M
9	FL 00972 3P 1 2P M
10	FL 00984 10P 8 1P M
11	FL 01870 5P 3 1P M
12	FL 01911 12P 5 1P M
13	FL 00440 47P 5 2P M
14	FL 00443 33P-4 3P M
15	FL 00477-45P 1 3P M
16	FL 00459 21P 2 2P M
17	FL 00459 21P 11 2P M
18	FL 00459 27P 3 3P M
19	FL 00470 29P 5 2P M
20	FL 00470 29P 7 3P M
21	FL 00478 29P 5 1P M
22	FL 00518 14P 15 3P M
23	FL 00518 23P 11 2P M
24	FL 00520 14P 1 3P M
25	FL 00529 1P 3 2P M
26	FL 00530 7P 7 1P M
27	FL 00530 7P 7 2P M
28	FL 00530 7P 7 3P M
29	FL 00585 26P 1 2P M
30	FL 00593-4P 1 1P M
31	FL 00593-4P-4 2P M
32	FL 00593 6P 1 3P M
33	FL 00593 6P 8 1P M
34	FL 00595 5P 8-4P M
35	FL 00595 9P 6 3P M
36	FL 00595 18P-4 2P M

Table 7 Percentage of Blast Resistant Lines in the Cross of the Commercial Rice Cultivar Bg 90 2/*Oryza rufipogum* Evaluated under Greenhouse and Field Conditions

Cultivar / Wild Species	Isolate 1 Bg 90 2	Isolate 2 Amistad 82	Field Reaction Leaf	Field Reaction Neck
Bg 90 2	R I	S	S	S
Oryza rupipogum	S	R I	S	S
Cross Bg 90 2/*O rufipogum* (204 F_6 lines)	4 9 / R	84 3 / R	59 8 / R	8 3 / R
Line CT 13946 –1 1 M M 2 Resistant in greenhouse and field evaluations				

R=Resistant I=Intermediate S=Susceptible

Reference

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

- **Identification of Molecular Markers Associated with the Blast Resistance Genes Pi 1 Pi 2, Pi 11 and their Incorporation into Commercial Rice Varieties Through Backcrossing and Marker Assisted Selection (MAS)**

F Correa, G Prado F Escobar C P Martinez Didier Tharreau (CIRAD) M Vales

Abstract

Blast susceptible varieties are often chosen by farmers because they have high yields and grain quality Since blast resistance is often broken down in a relatively short time these varieties become less desirable to the farmer Incorporation of blast resistance to these varieties would make these varieties by more cost effective and ecologically sustainable The combination of blast resistance genes Pi 1 Pi 2 and Pi 11 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

Farmer adopt rice varieties that have high yields and excellent grain quality The characteristic such as resistance to diseases are highly desirable but not enough to make a variety successful Inconsistant yields because of diseases is 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 cultivars which still play an important role in the economy of many rice farmers and regions of Latin America The resistance genes to be incorporated into the commercial varieties will be Pi 1 Pi 2 and Pi 11 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 Suitable rice breeding populations carrying the resistance genes Pi 1 Pi 2 and Pi 11 have been developed for the identification of molecular markers associated with the resistance genes These markers will be used for the introgression of these genes into the background of the rice commercial cultivars

Materials and Methods

A genetic study to confirm the presence of the three resistance genes Pi 1 Pi 2 Pi 11 was conducted in the cross between the near isogenic lines C 101 LAC and C 101 A 51 sources of the genes Two hundred eighty three F_2 plants and their corresponding F_3 lines were developed and used in greenhouse inoculations Four blast isolates carrying the corresponding avirulence genes avr Pi 1 avr Pi 2 (two isolates) and avr Pi 11 were used for the inoculation of the F_2 and F_3 lines The latter generation was inoculated in three replications of 10 plants per replication per line All plants were inoculated at 21 days after planting by spraying a spore suspension of $5x10^5$ spores/ml Inoculated plants were incubated before evaluation under high relative humidity for 15 days The plants were given a high nitrogen rate equivalent to 160 kg/ha to favoring blast development The most infected leaf of the individual plants were evaluated for percentage of leaf area affected and lesion type (1 4) DNA from each rice line was extracted and was used together with the phenotypic reaction to identify molecular markers (SCARs RAPDs microsatellites) associated with the three resistance genes One of the lines carrying the three resistance genes is being used in a backcrossing program for the incorporation of the three genes into 14 Latin American rice varieties These varieties are Fedearroz 2000 Colombia XXI Oryzica 1 and Fedearroz 50 from Colombia Epagri 108 Irga 409 Primavera and Bonanza from Brazil El Paso 144 from Uruguay and Argentina Cimarron from Venezuela Capirona from Peru Panama 1048 from Panama CR 1113 from Costa Rica and J104 from Cuba The molecular markers associated with the resistance genes are being used in each backcross for identifying the lines carrying the three genes

Results

Segregation of each resistance gene or the three resistance genes (Pi 1 Pi 2 Pi 11) in the 283 lines developed from the cross between the near isogenic lines C 101 LAC and C 101 A51 was as expected for three dominant and independent genes (Table 8) Near isogenic lines carrying any of the genes combination of any two genes and combination of the three genes were identified based on the artificial inoculations and are being advanced for future work on studies of the association of the corresponding avirulence genes with pathogen fitness Four lines were identified carrying the three resistance genes based on the greenhouse inoculations with the four blast isolates These lines were found to be resistant in the field at Santa Rosa in 2001 at both leaf and panicle stages One of these near isogenic lines carrying the three resistance genes is being used as donor of the three genes in a backcross program with 14 Latin American rice varieties Bulk segregant analysis using DNA of susceptible and resistant plants for each one of the genes is being used to identify molecular markers associated with the resistance genes So far the marker SCAR B 10 derived from a RAPD marker at the biotechnology unit (see annual reports of previous years) has been found to be associated with the resistance gene Pi 2 This marker separated perfectly the resistant (presence of a band) lines carrying the gene Pi 2 and the susceptible (absence of the band) lines without Pi 2 However the band present in the near

isogenic lines carrying Pi 2 was also present in other susceptible backgrounds lacking a functional Pi 2 gene This result suggests the need for identifying a molecular marker closer to the resistance gene Pi 2 based on the SCAR B 10 marker

Discussion

We have confirmed the presence of two different resistance genes in the near isogenic line C 101 LAC When developed this near isogenic line was reported to carry only the gene Pi 1 but there was found an additional resistance gene similar to a gene present in the rice cultivar Irat 7 (Didier Tharreau personal communication) which has been tentatively named as Pi 11 In their work they have called the corresponding avirulence gene as ace 1 which encodes a protein with similarity to a fungal polyketide synthases gene These type of proteins normally play a role in fungal metabolism and then a mutation at this gene could affect pathogen fitness (Leach et al 2001) This Pi 11 gene is also similar to the gene Pi zh that has been located on chromosome 8 The other resistance genes Pi 1 and Pi 2 are located on chromosomes 11 and chromosome 6 respectively The combination of the three resistance genes in a single near isogenic line conferred complete blast resistance in the field and greenhouse inoculations A set of near isogenic lines carrying the genes individually combinations of two genes and the combination of the three genes were developed These lines are being used to determine the effect of the different gene combinations on the development of blast We will also determine if the corresponding avirulence genes are associated with pathogen fitness According to our studies the avirulence gene to Pi 1 is highly conserved in the pathogen population (see Table 1 of activity 1) This suggests that this avirulence gene has lower rates of mutation to virulence and probably has an important role in fitness of the pathogen or in a conserved metabolic function On the other hand the avirulence gene for Pi 2 is present only in the genetic lineage SRL 5 This suggests that this avirulence gene has higher rates of mutation to virulence and that the resistance gene Pi 2 would be less durable This is confirmed by our studies as most isolates of the blast pathogen studied so far defeats the Pi 2 resistance gene The avirulence gene for Pi 11 seems to be highly conserved in the pathogen population suggesting that this gene would also be more durable than the resistance gene Pi 2

Future activities

Using the near isogenic line studies to determine the effect of the individual blast resistance genes (Pi 1 Pi 2 Pi 11) and their combinations on the development of blast are continuing The role of the corresponding avirulence genes (avrPi 1 avrPi 2 avrPi 11) on pathogenic fitness is being done to predict the durability of these genes The identification of suitable molecular markers associated with the resistance genes Pi 1 Pi 2 Pi 11 is continuing and will be used in marker assisted breeding backcrossing program as well as other breeding programs to obtain durable blast resistance

Table 8 Inheritance of Resistance to *Pyricularia grisea* in the F_3 Progeny of the Cross C 101 A51 x C 101 LAC

Isolate	Genetic Lineage	F_3 Resistant	F_3 Segregant	F_3 Susceptible	Expected F_3 Segrgation	X^2	Probability	Genetic Base of Resistance
Fanny 54	SRL 6	86	138	59	1 2 1	3 14	0 25 0 10	P_1 11 (1 dominant locus)
OY9 19 1	SRL 6	76	135	72	1 2 1	0 56	0 90 0 75	P_1 1 (1 dominant locus)
Isol 6-7 1	SRL 5	57	154	70	1 2 1	3 51	0 25 0 10	P_1 2 (1 dominant locus)
Isol 22 3 1	SRL 5	67	146	70	1 2 1	0 26	0 90 0 75	P_1 2 (1 dominant locus)

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

F Correa, C Martinez Jershon Lopez (KSU) Scot Hulbert (KSU) Robert Zeigler (KSU) Joe Tohme (BRU) Gerardo Gallego (BRU) Gustavo Prado Girlena Arcapa

Abstract

Blast resistance has been defeated in periods of 1 3 years after release of new commercial varieties An exception is the variety Oryzica Llanos 5 which has remained resistant to rice blast after more than 10 years of cultivation Understanding the genetic mechanism and identifying the resistance genes controlling the durable resistance of this variety is important for the development of systematic rice blast breeding strategies We are using RIL s for the evaluation and identification of molecular markers associated with the resistance genes in O Llanos 5 Several major genes have been identified using microsatellite markers

Introduction

Typically blast resistance has been overcome in periods of 1 3 years after the release of new commercial varieties However the resistance of the variety Oryzica Llanos 5 has been durable and remained stable under field conditions for more than 10 years Genetic studies have indicated the presence of at least four major genes controlling the resistance to various isolates of blast Based on the presence of avirulence genes in our blast populations we have inferred that the variety O Llanos 5 carries at least 8 major genes (Table 3) Studies of blast isolates recovered from the immediate parents of Oryzica Llanos 5 that included the characterization of their genetic structure and virulence suggest that the durable resistance is associated with the pyramiding of complementary resistance genes to the different lineages of the pathogen Since there are few examples of durable resistance understanding the basis of the durable resistance of Oryzica Llanos 5 should lead to the development of more systematic breeding strategies A study to identify and dissect the resistance genes present in Oryzica Llanos 5 was initiated in collaboration with Kansas State University

Materials and Methods

Almost 1000 recombinant inbred lines (RIL s) of the cross between the Oryzica Llanos 5 and the susceptible variety Fanny were produced Initially 120 lines were inoculated with different blast isolates representing the pathogen genetic lineages SRL 1 to SRL 6 from Colombia Inoculations and evaluations were performed at CIAT according to the methodology described previously DNA of each of the 120 lines was extracted at Kansas State University for molecular analysis and

microsatellites were used as potential markers to identify the resistance genes present in Oryzica Llanos 5 This is being done by using a set of molecular markers that systematically cover the rice genome and analyzing markers appearing to be associated to phenotypic reactions that are specific for each of the rice blast linages This should allow the tagging of the resistance genes to each genetic lineage of the pathogen

Results and Discussion

Preliminary results have been generated for chromosomes 4 5 6 8 11 and 12 Resistance genes to several of the blast isolates used in the inoculations have been associated with molecular markers that tag regions on chromosomes 8 and 6 These could be related to the genes Pi 11and Pi 2 but additional lines need to be evaluated to confirm these observations Other resistance genes appear to be on chromosomes 4 11 and 12 suggesting that the resistance in the cultivar Oryzica Llanos 5 is controlled by several major genes as suggested by other studies (see previous section of this report) More RIL s are being inoculated for a more detailed analysis

The preliminary results obtained to date suggest that several major resistance genes govern the durable resistance observed in the rice variety Oryzica Llanos 5 In order to be able to detect most resistance genes and QTL s (quantitative trait loci) present more of the RIL s are being evaluated using several replications to generate precise information on the quantitative effects of the resistance genes

Future activities

The preliminary results will be confirmed both by the testing of additional RILs and by more intensive evaluation to the different linages of selected RILs Microsatellites will continue to be used to identify and locate more blast resistance genes A group of near isogenic lines with known combinations of the blast resistance genes present in Oryzica Llanos 5 will be developed

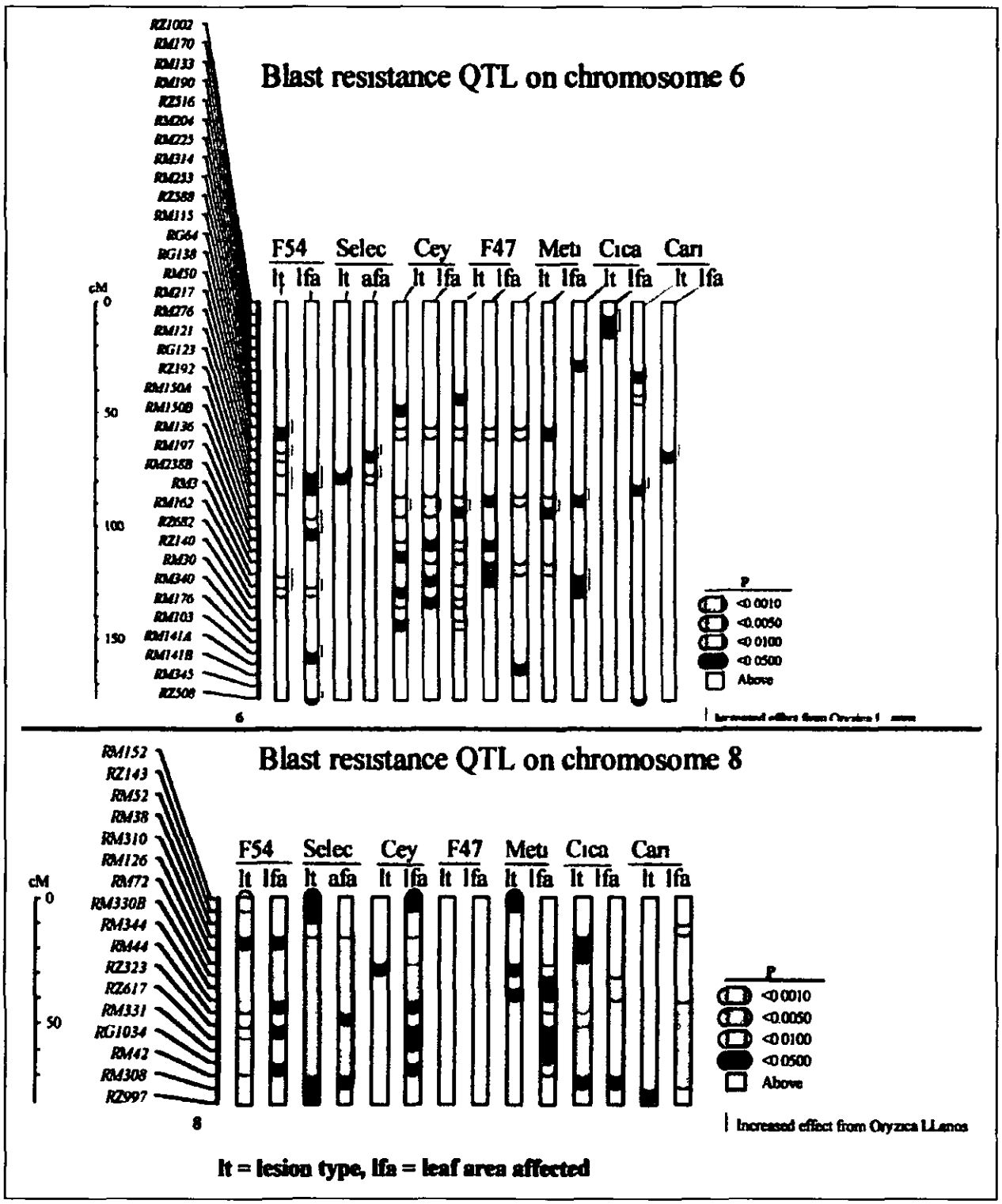

Figure 1 Blast Resistance Genes on Chromosomes 6 and 8 based on the Inoculation of Recombinant Inbred Lines with Seven Rice Blast Isolates

- **Evaluation of Breeding Populations Incorporating Complementary Resistance Sources to Blast in Greenhouse and Field Experiments 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 selected for resistance after four 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 to associate the stability of blast resistance and the blast 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 the 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 generation is not known We have initiated a long term study to associate the stability of blast resistance and the blast reaction of F_2 populations 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 We expect to generate information that helps rice breeders for making decisions that increase the chances of selecting lines with stable blast resistance

Materials and Methods

A study to evaluate the stability of blast resistance was initiated in 2000 Each of 45 parents were used in at least 1 and up to 6 crosses The parents exhibited a resistance intermediate or susceptible reaction Triple crosses involving three resistant parents (greenhouse and field evaluations) three susceptible parents and crosses where the predominant F_2 family field reaction was either susceptible segregating (equal amount of F_2 susceptible/resistant plants) or resistant F_2 plants were made In the F_2 generation the resistant plants were selected during both 2000 and 2001 The F_3 lines derived from F_2 resistant plants selected in 2000 were evaluated in 2001 Each line was planted in 10 rows 2m long Two plants per row were randomly selected and were

evaluated for leaf blast at 25 32 and 39 days after planting and 25 and 32 days after flowering for panicle blast Three to five F_3 resistant plants were selected from each line for field evaluations in 2002 Studies of the stability of the blast resistant reaction will be initiated in replicated trials in the F_4 generation and continue over more advanced generations on a year basis until the number of lines losing the resistance reaches a plateau Parents used in the crosses under study are being planted for detailed observation of their blast reaction and for collection of blast isolates to be used in greenhouse studies

Results

A total of 309 F_2 blast resistant plants from 69 crosses and 169 families were selected in year 2000 (Table 9) The resistant plants were selected from triple crosses involving three resistant parents (greenhouse and field evaluations) or three susceptible parents Other resistant plants were selected from crosses where the predominant F_2 family field reaction was either susceptible segregating (equal amount of F_2 susceptible/resistant plants) or resistant F_2 plants (Table 9) Evaluation of the F_3 lines derived from F_2 resistant plants selected in 2000 yielded more resistant lines (77%) when selections were made from the F_2 populations that were rated as resistant to blast (Table 9) The lowest percentage of resistant F_3 lines (4%) was obtained from F_2 families where the three parents had exhibited a susceptible blast reaction (Table 9) Base on the number of F_3 lines selected the field reaction of the F_2 populations was a more important selection criteria than the apparent reaction of the parents involved in the crosses Those crosses involving three resistant parents were selected on the blast reaction in only one field or greenhouse observation It is necessary to corroborate the reaction of the parents as well as the reaction of the F_2 populations of those crosses to have a more precise conclusion In this study 36% of F_3 resistant lines were identified from selections of F_2 populations where susceptibility was predominant (Table 9) The selected resistant F_4 plants will be planted in 2002 in replicated trials and evaluations will continue on more advanced generations to compare the stability of the blast resistance and its association with the blast reaction of the original F_2 giving origin to that line During 2001 additional crosses and families were evaluated and the resistant F_2 plants were selected The plants will undergo addition evaluations in 2002

Discussion

Our hypothesis in this study is to demonstrate that those lines originating in crosses where the F_2 shows 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 We also predicted that those advanced lines originating from F_2 resistant plants selected within crosses where F_2 susceptible plants predominated will be less stable

Our reasoning behind this hypothesis is that F_2 population exhibiting a predominant number of resistant plants carries a larger number of different resistance 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 therefor 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 the hypothesis is correct breeder should rate the F_2 populations and eliminate those crosses where susceptible plants predominate This would allow their effort to be concentrated on those crosses where there is greater probability of selecting stable blast resistant rice lines

It should also be important to characterize well the blast reaction of the parents used in the selected crosses in order to associate their reaction with the observed blast reaction of the F_2 population as a indicator of the future stability of the resistance in the selected lines

Future activities

This study is in progress and the evaluation and selection of blast resistant lines will continue over time for determining their stability The characterization of the blast reaction of the parents used in the selected crosses will continue in order to determine their role in the stability of the resistance

Table 9 Long Term Study on the Stability of Blast Resistance based on F_2 Resistant Plants Selected from Different Populations

Population Cross/Family	Crosses (No)	Families (No)	Resistant F_2 Plants Selected in 2000 (No)	Evaluation of F_3 lines in 2001		
				Resistant No (/)	Segregating No (/)	Susceptible No (/)
Progenitors Selected						
R/R//R	13	27	53	12 (23)	34 (64)	7 (13)
S/S//S	8	15	28	1 (4)	1 (4)	26 (92)
F_2 Family Field Reaction in 2000						
Susceptible	27	50	89	32 (36)	37 (42)	20 (22)
Segregating	27	47	79	45 (57)	27 (34)	7 (9)
Resistant	18	30	60	46 (77)	12 (20)	2 (3)
TOTAL	**69**	**169**	**309**			

Table 10 Selection of Blast Resistant Plants Based on F_2 Family Field Reactions at Santa Rosa in 2001 for Studies on the Stability of Blast Resistance

Cross/Family	Crosses (No)	Families (No)	Resistant F_2 Plants Selected (No)
F_2 Family Field Reaction			
Susceptible	42	53	109
Segregating	48	50	93
Resistant	36	49	96
TOTAL	**87**	**152**	**298**

OUTPUT 2 CHARACTERIZING RICE PESTS AND THE GENETICS OF RESISTANCE

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

- **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 Vales E Tulande J P Dossman J Garcia F Rodriguez V H Lozano M Triana V Kury M C Duque

Abstract

A new 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* subsp Indica upland 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

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 (Vales 1983 and 1987) To facilitate the genetic recombination a recessive gene of male sterility is used (Singh and Ikehashi 1991)

The developed scheme of recurrent selection has three parts (Vales Rice Project Annual Report 1998) These include the selection of complete resistance and the selection for the partial resistance and other agronomic characters The third part of the method involves the genetic recombination that maintains the population s variability and a high frequency of the male sterile gene to allow further cycles of recurrent selection

Materials and Methods

The population PCT 6 of *O sativa* subsp Indica upland rice went through two cycles of recurrent selection for rice hoja blanca virus by Chatel (see CIAT annual report 2000) Additional cycles of recurrent selection were then done (Vales Rice Project Annual Report 1999) The origin of the material used in the trials is described in the follow paragraphs

Selection for the Complete Resistance (A as First Semester, B as Second Semester)

1997B and 1998B There were two cycles of selection in S_1 lines in greenhouse The genetic progress of complete resistance due to the first selection cycle for this resistance was considerable with an important increase of the percentage of S_1 lines resistant to strains of the different rice blast lineages

1999A A cycle of plant parasite reciprocal selection in S_1 plus S_3 in field

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 3 S_1 lines (equivalent of F_2 population) were obtained with complete resistance to all the strains that were tested (Rice Project Annual Report 2000) The remaining seeds of the S_1 selected lines constitute the material used for the another cycle of genetic recombination during 2001

Selection for the Partial Resistance to Rice Blast Disease, Pest Tolerance, and Other Agronomic Traits

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* (Rice Project Annual Report 1998)

1999B The S_0 obtained with the recombination was planted The information on the S_1 or S_3 origin of the S_0 plants is stayed 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 are the S_1 lines

2000B Seeds of S_2 lines without complete resistance to a chosen strain were obtained It is the material used in the selection for the partial resistance to rice blast disease pest tolerance and other agronomic traits during 2001

Genetic Recombinations

1998A Recombination in S_1 plus S_0 after the selection in greenhouse for the complete resistance in S_1
1999A Recombination in S_1 plus S_3 and incorporation of S_0 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

Selection for the Partial Resistance to Rice Blast Disease, Pest Tolerance, and Other Agronomic Traits in 2001

The trial was carried out in Santa Rosa experimental station Villavicencio Meta Colombia during the first semester 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) A *Panicum maximum* border was used to isolated the trial The spreader rows consisted of Oryzica 1 inoculated with dry leaves of plants infected with the strain Fanny 54 (10 g of dry leaves / m^2 of spreader)

Genetic Recombination in 2001

In the experimental station of Palmira Valle Colombia during the first semester 2001 5 000 were sowed in field isolated using a plastic barrier There were approximately 2 500 S_3 S_1 plants and 2 500 S_0 plants The recombination lines were harvested from the male sterile plants

Results

Selection for the partial resistance to rice blast disease, pest tolerance and other agronomic traits in 2001

The genetic progress for partial resistance in the field seems significant but is too early to make any conclusion The statistical analyses are being done for partial resistance and for the other agronomic traits

Seeds were harvested from the PTC 6 population that underwent a cycle of genetic recombination in 2001

Discussion

The improvement of this upland rice populations for Colombian Llanos will continue But the demonstrative methodological investigation of this new recurrent selection scheme for the partial and complete resistance to rice blast disease pest tolerances and other agronomic traits was completed Therefore the following part of the work will be focused on the publication of the results and on obtaining varieties using the materials generated during these trials

This new selection scheme will be applied to other recurrent populations with narrow genetics base These include PCT 14 an upland rice population for the cold hillsides PCT 16 for lowland rice population for Colombian Llanos and PCT 18 an upland rice population for tropical hot area (Vales Rice Project Annual Report 2000)

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- **Study of QTLs of Partial Resistance to Rice (*Oryza sativa*) Blast (*Magnaporte grisea*) Disease in Fixed Progenies of IR 64 / Azucena**

M Vales J Dossmann E Tulande J Garcia F Rodriguez V H Lozano

Abstract

Controlled inoculations in greenhouse showed that no known Colombian rice blast isolates can overcome the complete resistance of many of the IR 64 / Azucena progenies There were sufficient resistant lines to allow the study of partial resistance QTLs in the field A preliminary evaluation demonstrated that these IR 64 / Azucena progenies segregated for partial resistance Therefore these progenies and methods are adequate for the study of partial resistance QTL when the number of compatible lines will be enough

Introduction

The final objective of these studies is the identification of molecular markers for the partial resistance to rice blast disease of IR 64 and/or Azucena, and the use of these markers in selection To study partial resistance field evaluations were needed to distinguish between lines with complete and partial resistance (Vales 1983 1987)

Materials and Methods

Seeking Compatible Strains of *Pyricularia oryzae* (*Magnaporthe grisea*) with Fixed Rice (*Oryza sativa*) Progenies of IR 64 / Azucena

Hundred four fixed IR 64 / Azucena progenies provided by CIRAD (Huang *et al* 1994) and the variety Fanny as susceptible check were used in this study The 13 isolates used come from the 7 lineages identified in the Colombian Llanos (Correa Victoria and Zeigler 1993 Flor Payan 1998) The trial was carried out in greenhouse of the experimental station of Palmira Valle Colombia during the end of the second semester 2000 Each line and check was sown with 20 seeds per row After 18 days the plants were inoculated by aspersion using 40 ml of a suspension of 5 x 10^5 conidia/ml After 12 days in dew chamber the notation of the symptoms was made Progeny from the 400 fixed lines were evaluated using all 13 rice blast isolates

Evaluation of Partial Resistance to Rice Blast Disease of IR 64 / Azucena Progenies

Thirty five IR 34 / Azucena fixed lines without complete resistance to the isolate Fanny 54 were used for this study The isolate Fanny 54 is part of the lineage SRL 6 (Correa Victoria and Zeigler 1993) The susceptible variety Oryzica 1 specifically susceptible to the strains of the lineage SRL 6 was used to form the spreader

The trial was carried out in Santa Rosa experimental station Villavicencio Meta, Colombia during the first semester 2001 The trial design was in Federer s blocks of 20 lines and 5 checks Two rows of 5 m were sown at a density of 19 kg/ha) The trial was isolated by a *Panicum maximum* border The spreader was inoculated with dry leaves of plants infected in greenhouse with the isolate Fanny 54 at a rate of 10 g of dry leaves / m^2 of spreader

Results and Discussion

Seeking Compatible Strains of *Pyricularia oryzae* (*Magnaporthe grisea*) with Fixed Rice (*Oryza sativa*) Progenies of IR 64 / Azucena

In the first series of 104 IR 64 / Azucena fixed progenies 35 lines were without complete resistance to a same strain What is insufficient for a QTLs analysis of partial resistance to rice blast disease However a first preliminary evaluation of the level of partial resistance was carry out to make sure that there was enough variability of the blast resistance (see trial 2)

Evaluation of partial resistance to rice blast disease of IR 64 / Azucena progenies

Although the statistical analysis of the data is not complete it is possible to observe the segregation for partial resistance using the criteria of the percentage of infected leaf area (Figure 1) the percentage of infected plants (Figure 2) and the percentage of panicles with neck blast (Figure 3) There are a sufficient number of lines without complete resistance that the identification of QTLs for the partial resistance will be possible using the progenies of this cross in field trails and molecular analyses

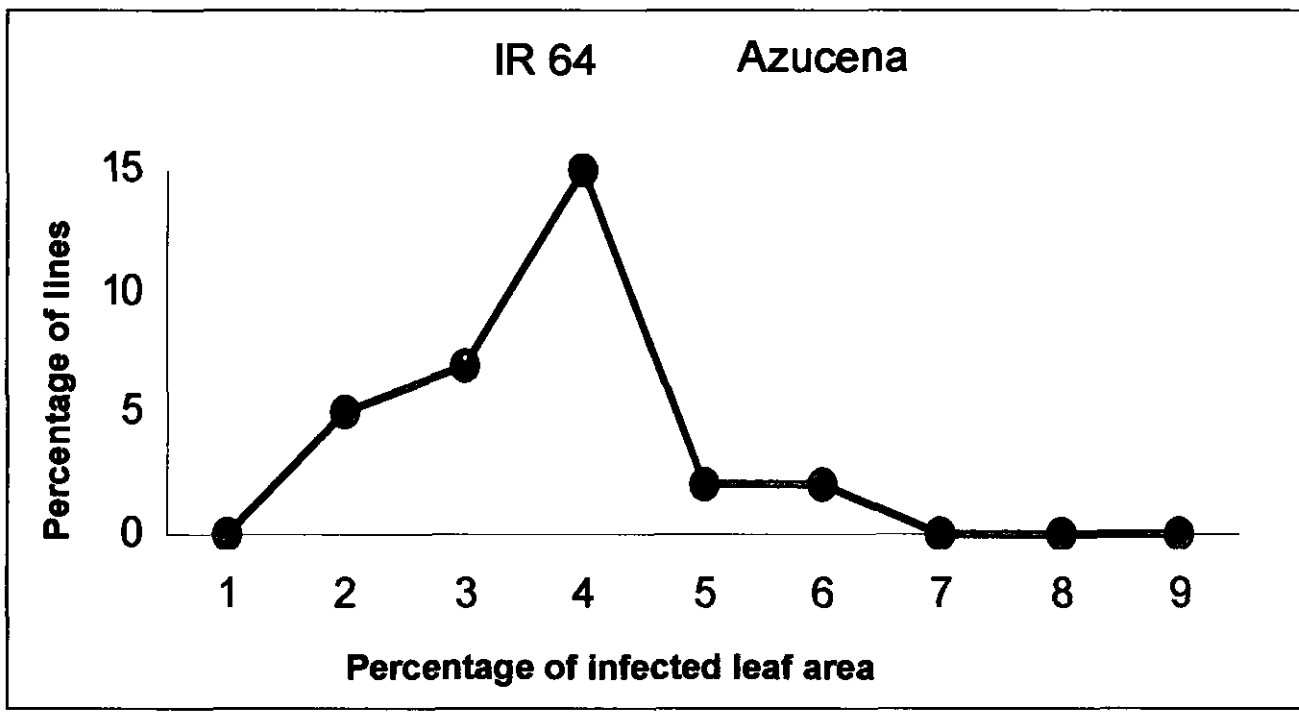

Figure 1 Leaf Blast in IR 64 / Azucena Progenies

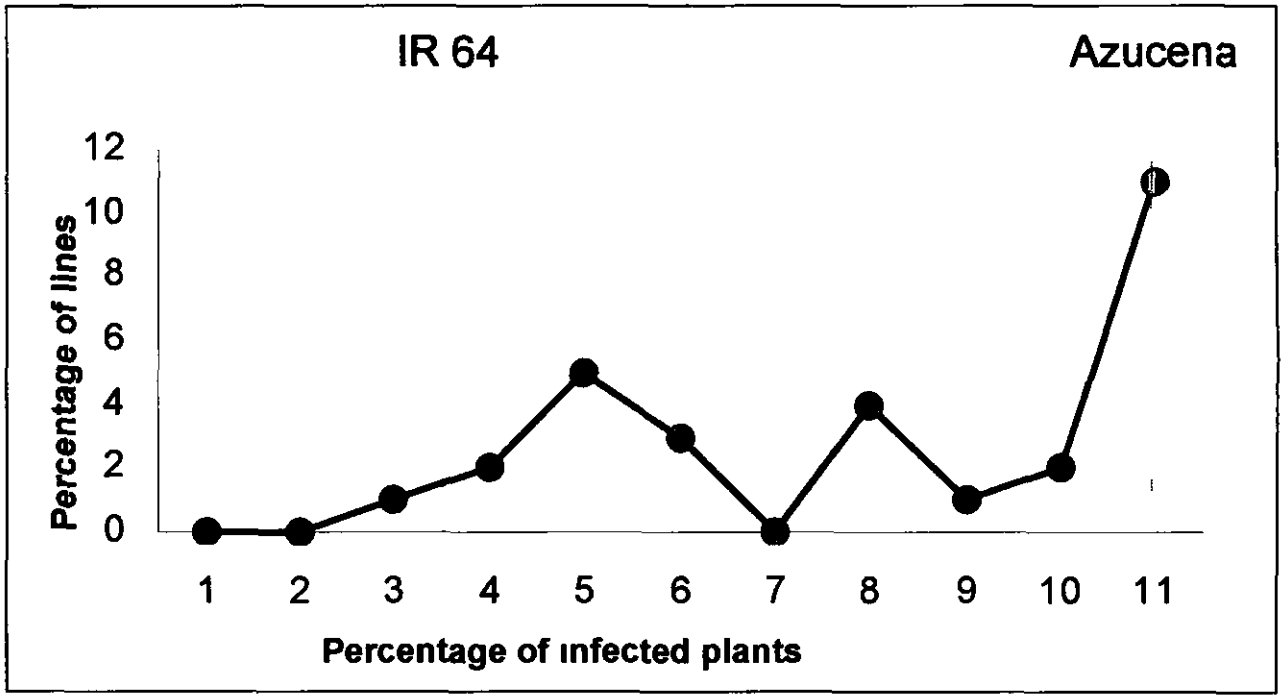

Figure 2 Plants Infected by Blast in IR 64 / Azucena Progenies

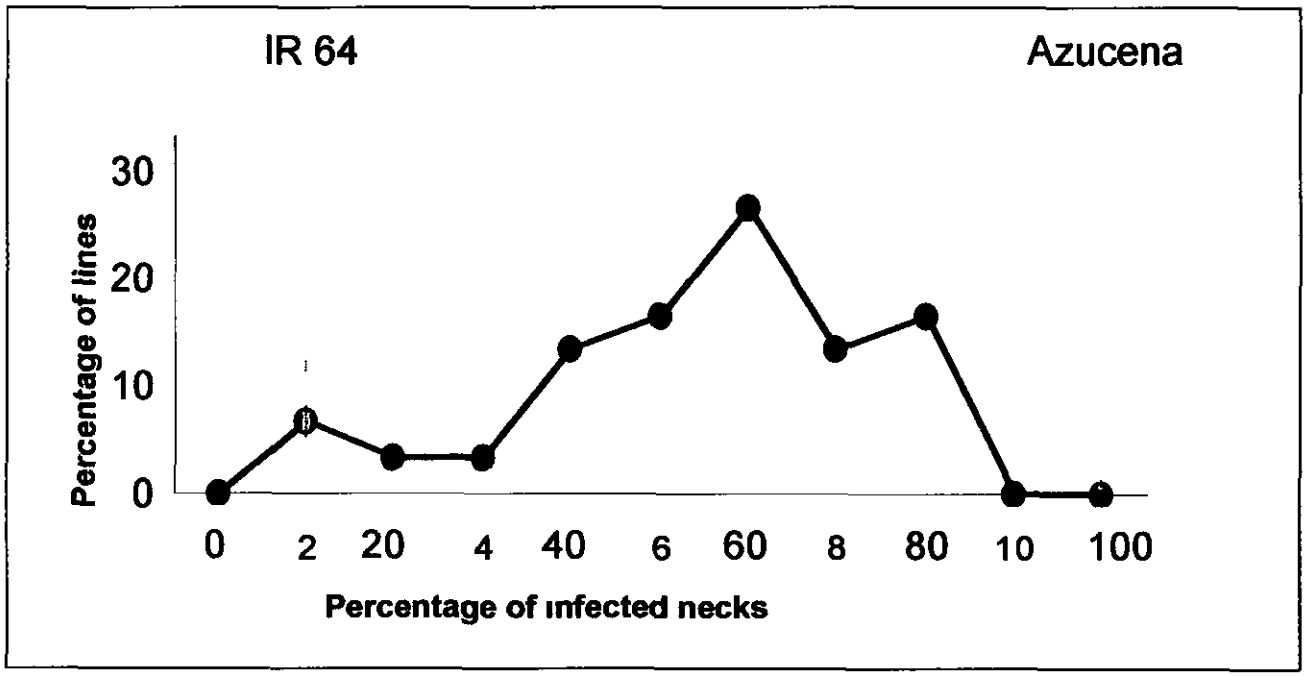

Figure 3 Neck Blast in IR 64 / Azucena Progenies

Perspectives

The introduction of 149 lines more was made to try to find a sufficient number of compatible lines with the same isolate We are looking for at least 2 compatible isolates and no less than 50 lines in order to discard QTLs of specific partial resistance (Vales Rice Project Annual Report 1998)

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- **Application of Back Recurrent Selection to Obtain and Transfer Resistance to Rice Blast**

Abstract

Back recurrent selection (Vales 2000) is a new method for the introgression of polygenic traits to improve elite varieties This is the first use of this method to transfer partial resistance to rice blast disease into selected commercial varieties It has been proposed that no *Pyricularia oryzae* (*M grisea*) strain can possess genes of virulence av 1 av 2 and av 11 at the same time If this hypotheses is correct the association of the corresponding major resistance genes Pi 1 Pi 2 and Pi 11 should produce a rice line with durable resistance to rice blast The preliminary results of the study of isolate variety compatibility that is needed to begin the genetic improvement of elite varieties using this strategy of incompatibility of virulences

- **Back Recurrent Selection for the Partial Resistance to Rice (*Oryza sativa*) Blast (*Magnaporthe grisea*) Disease**

Introduction

Back crosses are adapted to introduce few new alleles into elite varieties But this method is inadequate for the introgression of polygenic traits So a new original method was imagined for the transference of polygenic characters the back recurrent selection The following activity is the beginning of the use of this new method to transfer partial resistance to rice blast disease into commercial varieties

Material

IRAT 13 and Moroberekan were used as the progenitors for partial resistance The commercial varieties to be enhanced come from different partners of different countries for different cropping types of rice (Table 1)

Table 1 Rice Commercial and Elite Varieties

Variety	Type	Country and Partner
Tropical lowland rice		
Couachi	Indica	French Guyana CIRAD CFR
Crete a Pierrot	Indica	Haiti MARNDR/CRDA
Colombia XXI	Indica	Colombia FEDEARROZ
Fedearroz 50	*Indica*	Colombia FEDEARROZ
Fedearroz Victoria I	*Indica*	Colombia FEDEARROZ
Fedearroz 2000	*Indica*	Colombia FEDEARROZ
Fedearroz Victoria II	*Indica*	Colombia FEDEARROZ
Cimarron	Indica	Venezuela DANAC
Palmar	*Indica*	Venezuela DANAC
Fonaiap 1	*Indica*	Venezuela DANAC
Minghui No 63	*Indica*	China YAAS
Minghui No 72	*Indica*	China YAAS
Dianlong No 201	*Indica*	China YAAS
Yunhui No 290	*Indica*	China YAAS
Guichao No 2	*Indica*	China YAAS
Temperate lowland rice		
Artiglio	Indica	France CIRAD CFR
Merveilleux	Indica	France CIRAD CFR
Basmati C621	*Basmati*	France CIRAD CFR
Dedalo	*Japonica*	France CIRAD CFR
Dorella	*Japonica*	France CIRAD CFR
Drago	*Japonica*	France CIRAD CFR
Estrela	*Japonica*	France CIRAD CFR
Idra	*Japonica*	France CIRAD CFR

Dedalo x Miara	*Japonica*	France CIRAD CFR
Kulon	*Japonica*	Russia CIRAD CFR
Sandora	*Japonica*	Hungria CIRAD CFR
Oro	*Japonica*	Chile INIA
Lowland rice of high altitude		
Latsibavy	*Japonica*	Madagascar CIRAD FOFIFA
CIRAD 407	Japonica	Madagascar CIRAD
CIRAD 408	*Japonica*	Madagascar CIRAD
Dianyu No 1	*Japonica*	China YAAS
Xinan 175	*Japonica*	China YAAS
Yunjing No 9	*Japonica*	China YAAS
Yunjing No 135	*Japonica*	China YAAS
Yunjing No 136	*Japonica*	China YAAS
Hexi No 35	*Japonica*	China YAAS
Hexi No 39	*Japonica*	China YAAS
Hexi No 40	*Japonica*	China YAAS
Hexi No 41	*Japonica*	China YAAS
Hexi No 42	*Japonica*	China, YAAS
Dianxi No 1	*Japonica*	China YAAS
Dianxi No 4	*Japonica*	China YAAS
Cujing No 17	*Japonica*	China YAAS
Cujing No 22	*Japonica*	China YAAS
Tropical upland rice		
Progresso	*Japonica*	Brazil EMBRAPA
Upland rice for hillsides of altitude		
IRAT 379	Japonica	Madagascar CIRAD FOFIFA
IRAT 380	*Japonica*	Madagascar CIRAD FOFIFA
CIRAD 391	*Japonica*	Madagascar CIRAD FOFIFA
CIRAD 392	*Japonica*	Madagascar CIRAD FOFIFA
CIRAD 393	Japonica	Madagascar CIRAD FOFIFA
CIRAD 394	*Japonica*	Madagascar CIRAD FOFIFA
CIRAD 409	*Indica / japonica*	Madagascar CIRAD FOFIFA
PRA 659	*Indica / japonica*	Colombia CETEC UMATA

Male sterile plants from recurrent populations with narrow genetic base were used

Method

The first step was to make to crosses of each commercial varieties with male sterile plants

Result and discussion

Some of the crosses with the male sterile plants and the commercial varieties have been made After the seeds of these crosses are harvested they will be crossed with each of the progenitors for partial resistance The progenies of each of the varieties will be evaluated for partial resistance in the field and the best lines will be recombined using the male sterility During this genetic recombination the corresponding commercial variety will to be added and this acts as a

backcross This allows the original plant type of the corresponding variety and the incorporation of partial resistance to rice blast

Perspectives

After an evaluation of the efficiency of this method the obtained varieties will be enhanced using the incompatibility of virulences The use of both strategies and methods should increase the likelihood of the resistance durability In the case of both strategies molecular markers that are develop will be incorporated it facilitate the selection

- **An Application of the Strategy of Incompatibility of Virulence**

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Introduction

In the case of rice blast disease all the known complete resistance genes are monogenic and specific i e an isolate can overcome this single gene resistance It has been proposed that associations of resistance genes could confer a more durable resistance The Pi 1 Pi 2 and Pi 11 plant resistant gene have associated with durable resistance (see the section in this annual report)

The genetic demonstration of the gene for gene interaction between the complete resistance of rice and *Pyricularia oryzae* virulence was carried out (Silue *et al* 1992 Tharreau *et al* 1993) To overcome the resistance due to the three genes i e alleles an isolate would need to have the three corresponding virulence alleles So the hypothesis to explain the durability of the complete resistance due to the effect of the three genes association is there are biological impediments that prevent an isolate from carrying the three corresponding alleles of virulence The strategy using this model is named incompatibility of virulence

Material and Methods

The commercial varieties to be enhanced come from different partners of different countries for different cropping types of rice (Table 1) The rice blast isolates chosen will allow each one of the 3 genes Pi 1 Pi 2 or Pi 11 (Table 2) to be analyzed individually

Table 2 Isolates Used to Evaluate the Presence of the Major Resistance Genes Pi 1, Pi 2, and Pi 11 (Isolate and Information from F Correa and D Tharreau)

strain	Resistance gene		
	Pi 1 C104Lac	Pi 2 C101A51	Pi 11 IR 1529
CL3 1 24		+	+
CL3 1 25		+	+
CL3 1 36		+	+
CL52	+		+
CL53	+		+
CL65	+		+
Fanny 54	+	+	

+ The strain cans overcome the gene effect i e symptom apparition
The strain cans not overcome the gene effect

Methods

Each isolate was inoculated on the test varieties to study their compatibility This activity began in greenhouse of the experimental station of Palmira, Valle Colombia, during the first semester 2001 Each line and check was sown with 20 grains per row After 18 days the plants were inoculated by aspersion with each one of the isolates (40 ml of a suspension of 5 x 10^5 conidia/ml by 30 x 40 cm) After 12 days in dew chamber plants were rate for symptoms

Results

The inoculations and the analysis are in progress

Discussion and perspectives

The major resistance genes Pi 1 Pi 2 and Pi 11 will be introduced into commercial varieties using a biological assay to confirm their presence The progenitor source of the 3 resistance genes is a line C101A51/C101LAC made by Fernando Correa (personal communication) To make this strategy more efficient marker aided selection for these 3 resistance genes is needed Studies of these markers are be done for Pi 1 Pi 2 (F Correa personal communication) and Pi 11 (D Tharreau personal communication)

After an evaluation of the efficiency of this method selected lines will be enhanced using the back recurrent selection for partial resistance (see above) The use of both strategies and methods could increase the likelihood of the resistance durability

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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*

- **Understanding the Genetics of Resistance in Rice to *Tagosodes orizicolus* and Rice Hoja Blanca Virus**

L Calvert R Meneses I Lozano and M Triana

Abstract

To systematically breed rice varieties to be resistant to *T orizicolus* and rice hoja blanca virus (RHBV) a better understanding of the type of traits associated with resistance and their genetics is needed Ten rice varieties were selected on the basis of their reactions to *T orizicolus* and RHBV These varieties were tested for the preference of *T orizicolus* to settle and oviposit on them The planthoppers preferred Cica 8 IR 8 Oryzica 1 and Fedearroz 2000 Fedearroz 50 was the least preferred variety and the planthoppers had the shortest longevity The same varieties were tested in the field for their reaction to RHBV by infesting them with viruliferous planthoppers at different dates Although the pressure was extremely high and it was not possible to quantify differences between the intermediate resistant varieties Fedearroz 2000 was clearly superior to all of the varieties including Colombia 1 which is a common source of resistance to the virus Using the information on the resistance of these ten varieties a study was started to mark resistance genes to both the planthopper and virus The use of RAPDs has led to some potential markers Crosses are being made between these ten varieties to better understand the genetics of resistance and identify molecular markers associated with resistance genes both to the planthopper and RHBV

Introduction

Both rice and *T orizicolus* are hosts to rice hoja blanca virus There have been studies done that suggest that two or three genes are involved for resistance to the planthopper and that a similar number are needed for resistance to the virus Resistance to the planthopper is complex Both antibiosis and antixenosis are known to be classes of resistance that can change the planthopper s settling preference longevity oviposition as well as other traits of the life cycle Plant resistance to RHBV is even more complex since it is an interaction of the plant virus and the vector RHBV is not mechanically transmitted and therefore it is not easy to separate resistant to the vector from that to the virus

Varieties including Makalioka IRAT 124 (Jennings & Pineda 1970 Orellana 1981) and Fedearroz 50 (Pardey 2000) have an antibiotic effect Antibiosis is considered to be unstable and is undesirable since it can accelerate the development of biotypes of the insect The evaluation for resistance to *T orizicolus* is done in the greenhouse using a planthopper mechanical damage scale The screening rates the lines for mechanical damage but does not distinguish between types of resistance and varieties with antibiosis are sometimes selected using this method It consumes much space and time since it requires the maintenance of a colony of the planthopper the propagation of the test plants as well as the infestation and rating of the plants The method rates lines as resistant intermediate or susceptible The intermediate lines tend to be the least consistent in their rating and this is attributed to the effect of the neighboring lines Settling and feeding preference on the lines being tested influences the lines in the test but since the reaction of most of the rice lines are unknown it is not possible to arrange the varieties by expected reaction in order to minimize the effect of mixing resistant and susceptible lines

The reaction of varieties to RHBV depends on the number of insects that are used to infest the plants their aggressiveness and the virulence of the colony the number of days that the plants are exposed to the vector and the plant age at the time of the infestation The plants are evaluated using a rating scale for the reaction of the variety to different levels of infestation as well as different dates of infestation Since these are field experiments that estimate the number aggressiveness and percentage of virulence of vectors in the colony the reaction of the test varieties is critical to interpreting the results This type of experiment is used to screen large numbers of varieties It consumes many resources to maintain a *T orizicolus* colony with more than 70% virulent insects as well as the field preparation and evaluation

To better understand the types of resistance this study combines in depth testing of selected lines for their reaction to *T orizicolus* and to RHBV In addition these same lines are being evaluated using molecular markers The molecular markers will then be correlated with specific types of resistance As markers are developed and the genetics of resistance is better understood breeding for resistance should become more systematic and cost effective It should also be possible to exclude antibiosis in future varieties by eliminating antibiotic progeny at an early stage of the breeding process This report describes field and greenhouse evaluations of resistance to the planthopper the virus and preliminary results on using RAPDs to identify molecular markers associated with resistance genes

Materials and Methods

The core varieties Ten rice varieties were selected for their reaction to *T orizicolus* as well as their ability to cross with the other varieties Bluebonnet 50 IR 8 CT10871 1 CA 1 M (WC 352) IR65598 27 3 1 (WC 366) and Colombia 1 are varieties that are susceptible to the planthoppers Oryzica Llanos 5 Fedearroz 2000 and CICA 8 have intermediate resistance Oryzica 1 is resistant while Fedearroz 50 has both antibiotic and antixenosis resistance to the planthopper (Pardey 2000)

Settling and oviposition preference studies The materials evaluated were Cica 8 Oryzica Llanos 5 Colombia 1 Oryzica 1 Fedearroz 50 IR 8 and Fedearroz 2000 Each experiment consisted of three blocks (each cage was one block) and four replications (plants) from each of the seven varieties At ten days of age the seedlings were transplanted in a square tray of 70 cm per side to form a circle according to a predetermined random pattern At 15 days after transplanting the tray was placed into a 1 m^3 cage and 200 female and 50 male *T orizicolus* newly emerged adults from a sequential colony grown on Bluebonnet 50 were liberated in the center of the circle The number of planthoppers per plant was counted daily for five days

The plants that were used in the study of settling preference were harvested at 10 days after the infestation with the 250 planthoppers The number of eggs per plant was determined using a stereomicroscope

Oviposition preference studies This study used the ten core varieties Each trial consisted of 10 plants/variety that were infested with recently emerged *T orizicolus* at 25 days after planting The plants were grown in pots that were covered with transparent acetate tubes that were 35cm in height and 5cm in diameter Each plant was infested with a pair of recently emerged adult planthoppers The plants were changed every seven days until the female died After the removal of the planthopper the plants were harvested and using a stereoscope the eggs were counted

Design of field experiment. The experiment plots were 1 m^2 that were distributed in a randomized design of 2 blocks each containing 3 replications Plants were infested with approximately 1 planthopper/plant in treatments of 7 14 21 and 28 days after planting using a colony where 70% of the individuals were able to transmit RHBV The evaluation for incidence of RHBV was made at 30 days after infestation using a scale of 0 9 which is based on the percentage of plants infected per row (IRRI 1996) The plots were harvested and the yield was converted to t/ha The statistical analysis was an ANOVA (SAS)

RAPD PCR analysis Total DNA was isolated from individual *T orizicolus* using a method developed for plants (Gilbertson et al 1991) with volumes of reagents appropriate for the small weight of the planthoppers The DNA was amplified using the polymerase chain reaction (PCR) The primers used were Operon series A B and C (Operon Alameda CA) The reactions were carried out using Taq polymerase and programmable thermal controllers (PTC 100 MJ Research Waltham MA) The reaction conditions for the first cycle were five min at 94 C two min at 40°C and three min at 72 C This was followed with 39 cycles of one min at 94 C 1 5 min at 40 C and two min at 72 C The PCR products were run in agarose gels stained with ethidium bromide and visualized using UV light

Results and Discussion

Settling and oviposition preference in open choice experiments On the first day after the liberation of the planthopper there was the least amount of variation in the percentage of individuals found on each variety By the second day the trends became fixed although they continued to become slightly more pronounced on the following days On the forth day there were no statistically significant differences in the preference of *T orizicolus* to settle on Cica 8 Oryzica 1 Fedearroz 2000 or IR 8 (Table 1) Within that group there was a higher percentage on CICA 8 and Oryzica 1 Based on the statistical analysis IR 8 and Fedearroz 2000 were not statistically different from Oryzica llanos 5 and Colombia 1 After the first day Fedearroz 50 was the variety least preferred by the planthoppers By the third day Fedearroz 50 had statistically significantly less planthoppers compared with all the other varieties This data is similar to a study where the planthoppers had the same affinity to settle on IR 8 and O llanos 5 and the least preference for Makalioka and Fedearroz 50 (Rice AR 2000)

Table 1 Settling Preference of *Tagosodes orizicolus* Between Seven Rice Varieties

	Percentage of Planthopper per Variety			
Variety	**Day 1**	**Day 2**	**Day 3**	**Day 4**
IR 8	13 9 c h[1]	14 4 b h	14 5 a h	15 0 a g
Cica 8	17 9 a c	18 4 a c	18 4 a c	19 1 a
Oryzica llanos 5	12 3 g i	12 5 f i	12 5 f i	12 6 f i
Colombia 1	11 0 h i	11 6 g i	12 1 g i	12 0 g i
Fedearroz 2000	14 9 a g	15 2 a g	16 2 a f	16 2 a f
Oryzica 1	17 3 a e	18 0 a c	18 7 ab	18 9 a
Fedearroz 50	12 6 f i	9 8 i	7 6 k	6 1 k

1 Different letter indicate that the results were significant at 0 05 level (Duncan alpha=0 05)

The preference for settling is also reflected in the number of eggs that were oviposited on each variety Again Cica 8 and Oryzica 1 were most preferred by *T orizicolus* but for this trait there were few statistically significant differences (Table 2) Colombia 1 was significantly different from Cica 8 and Fedearroz 50 Once again Fedearroz 50 was the least preferred variety

Table 2 Preference of *T orizicolus* for Ovipositing on Seven Rice Varieties in Open Choice Experiments

Variety	No of plants	Average number of eggs per plant[1]	Std Error
IR 8	48	154 8 ab	16 3
Cica 8	48	209 4 a	21 8
Oryzica llanos 5	48	143 3 ab	16 1
Colombia 1	48	122 7 b	14 8
Fedearroz 2000	48	148 4 ab	19 0
Oryzica 1	48	180 7 ab	17 8
Fedearroz 50	48	38 0 c	4 6

1 Different letter indicate that the results were significant at 0 05 level (Duncan, alpha=0 05)

In open choice experiments between these seven varieties there was more variation in the settling preference as compared to the oviposition preference This may reflect the relatively high density of planthoppers and their ability to move to a less preferred variety in order to lay their eggs Fedearroz 50 was the least preferred variety for both traits The lack of preference is probably because Fedearroz 50 has both antibiosis and antixenosis as resistance mechanisms (Rice AR 2000) Colombia 1 was the next lowest for both settling and ovipositing preferences but the differences were not statistically significant from most of the other varieties This could be due to Colombia 1 being a hybrid between Japonica and Indica The rest of the varieties were similar and there were no statistically significant differences

Oviposition preference in no choice experiments The ability of *T orizicolus* to oviposit was measured in no choice experiments Here the insects were placed on individual plants to measure the capacity of individual females The results (Table 3) were very different from the free choice experiments although Fedearroz 50 was notable for the low number of eggs laid upon it This experiment should be considered preliminary and the large standard error reflects the need for additional replications and possible modifications of this experiment Nevertheless many of the tendencies are similar to the open choice experiment The two lines WC 352 and 366 which were chosen because they are known to be highly susceptible to the planthopper and RHBV and are amenable as crossing parents did prove to be good hosts for the planthopper

Table 3 Preference of *T orizicolus* for Ovipositing on Ten Rice Varieties in Forced Feeding Experiments

Variety	Average number of eggs per plant
WC 352	191 6 a
WC 366	127 1 abc
Bluebonnet 50	85 7 bc
IR 8	65 0 bc
Cica 8	76 8 bc
Oryzica llanos 5	162 8 ab
Colombia 1	226 9 a
Fedearroz 2000	52 9 bc
Oryzica 1	31 4 c
Fedearroz 50	8 00 d

1 Different letter indicate that the results were significant at 0 05 level (Duncan alpha=0 05)

Understanding the mechanisms and genetics of the resistance is progressing The measurement of various factors in the life cycle of *T orizicolus* on different varieties of rice is allowing the plant resistance to be quantified Additional aspects of the life cycle on these varieties will be determined At the same time crosses between these varieties have been made and their F_2 populations will be used in the genetic and molecular marker studies This comprehensive approach of insect behavior and genetic analysis are traditional methods for understanding the inheritance of these traits This information will be useful in not only developing molecular marker but also in determining the function of the marker resistant traits

Field experiment for RHBV In the field experiment the colony was very aggressive and at the estimated level of one vector per plant only Fedearroz 2000 demonstrated a satisfactory level of resistance (Table 4) Fedearroz 50 Colombia 1 and Oryzica 1 are varieties rated as intermediate for resistance but in this experiment they were scored as 7 or above even at 21 and 28 days after planting This is a score that is normally considered susceptible to RHBV Since Colombia 1 is a known source of resistance to RHBV the disease pressure was so high that the resistance was not effective This experiment demonstrates that the common source of resistance to RHBV can be overcome by high level of inoculum pressure The performance of Fedearroz 2000 confirms that this variety has resistance that is superior to Colombia 1 which was the expected source of RHBV resistance Fedearroz 2000 is a new variety and still is not widely grown A major problem is the shattering of the grain but harvesting the grain before it becomes too dry can minimize this Fedearroz 2000 is the model for resistance to RHBV and in this study is the prime variety to be used to developing molecular markers

Table 4 Disease Severity of Ten Rice Core Varieties to RHBV at Four Dates of Infestation

	Infestation with ca 1 0 vector/ plant			
Rice lines	7 DAP[1] RHBV (0 9)	14 DAP RHBV (0-9)	21 DAP RHBV (0-9)	28 DAP RHBV (0 9)
WC 352	9 0 a	9 0 a	9 0 a	9 0 a
WC 366	9 0 a	9 0 a	9 0 a	9 0 a
Bluebonnet 50	8 2 a	9 0 a	9 0 a	8 2 a
IR 8	9 0 a	9 0 a	9 0 a	9 0 a
CICA 8	9 0 a	9 0 a	9 0 a	7 0 abc
Oryzica Llanos 5	9 0 a	9 0 a	9 0 a	9 0 a
Colombia 1	9 0 a	9 0 a	8 5 a	7 6 a
Fedearroz 2000	5 6 bc	5 4 c	3 2 d	2 6 d
Oryzica 1	9 0 a	9 0 a	7 4 ab	7 7 a
Fedearroz 50	7 3 ab	9 0 a	8 0 a	7 0 abc

DAP= Days after planting

Analysis of Molecular Markers To date 60 random primers have been tested and the are several potential molecular markers for resistance to both the planthopper and RHBV Using RAPDs unique bands have been associated with groups of varieties In the example shown a unique PCR product was found in the four varieties that have some resistance to *T orizicolus* but not in the other six varieties including five that are susceptible to the planthopper (Figure 1) These results are preliminary and must be confirmed by the test of F_2 populations of crosses between susceptible and resistant varieties These crosses have been made and F_2 populations will be tested next year In addition 240 more RAPDs are being tested As the results from the F_2 populations become available and the potential markers are confirmed additional techniques such as AFLP s and micro satellites will be used to map these marker to the rice genome

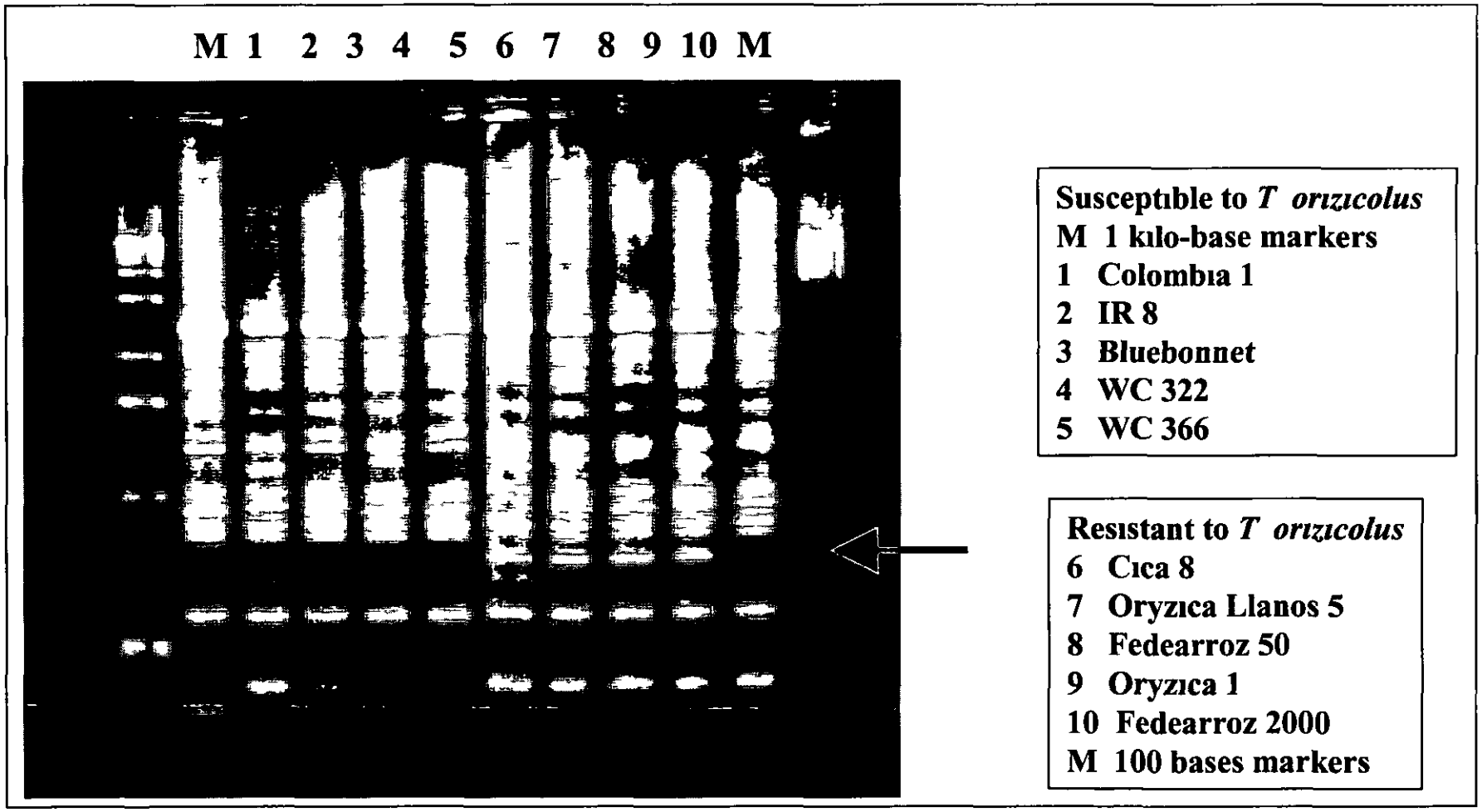

Figure 1 Potential Molecular Marker associated with resistance to *T orizicolus*

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- **Chemical Seed Treatments for the Control of *Tagosodes orizicolus***

R Meneses M Triana and L Calvert

Abstract

The effectiveness of several chemical insecticides applied as seed treatment was evaluated under semi controlled conditions Both fipronil and imidacloprid adequately controlled adult *Tagosodes orizicolus* at all doses evaluated when applied to pregerminated seed or when seed was immersed in insecticide solution Fipronil acts more quickly with seed pregerminated for 24 hours and then immersed from 30 to 60 minutes in insecticide solution The application of Confidor 350 (imidacloprid) diluted at 24 ml/l water to seed pregerminated for 24 hours and then placed in solution for 15 minutes was sufficient to control over 95% of *T orizicolus* present during the first 30 days after rice plant emergence

Introduction

Within integrated pest management (IPM) seed treatment is very important as a prophylactic measure because it not only controls pests but also relatively small quantities of pesticides are used It also reduces production costs and the risks for human health This method guarantees good germination and optimal plant density to obtain high yields

Fipronil belongs to a new class of insecticides called phenylpyrazols It disrupts the central nervous system of the insect by blocking the passage of chloride ions through the gamma aminobutyric acid (GABA) receptor therefore altering the central nervous system Normal doses of this insecticide causes insect death

Imidacloprid belongs to the class of the nitroguanidines acting as systemic or contact insecticide with prolonged residual effect It has two exclusive presentations one for seed treatment and the other a foliar spray

This study aims to determine the appropriate application doses immersion time and duration of impregnation of different insecticides for controlling *T orizicolus* under irrigated conditions

Materials and Methods

Comparing Pesticide Treatment on Dry and Pregerminated Seed Seed of rice variety Bluebonnet 50 was treated manually and evaluated according to different doses pregermination times of seed and duration of seed immersion in fipronil solution (6 ml/l water) Bluebonnet 50 seed was impregnated in water for 24 or 48 hours and then immersed in insecticide solution for 15 30 and 60 minutes Dry seed was also treated and a check (no application of insecticide) included Treated seed was then planted in pots and inoculated with 10 *T orizicolus* 11 days after planting Ten pots were used per treatment each with 5 plants and inoculated with 10 *T orizicolus* Determination of insect mortality began at 24 hours after inoculation with insects and continued for ten days

Comparing the Efficacy of Fipronil and Imadicloprid Imadicloprid comes in two types of formulations One formula is designed for better absorption through roots (Gaucho) and the other is formulated for foliar spray and absorption by leaves Application of chemical insecticides was similar except that seed was immersed in the insecticide solution for 60 minutes Each insecticide was applied at a dose of 6 ml/liter water Ten pots were used per treatment each with 5 plants and infested with 10 adult *T orizicolus* Insect mortality was assessed at 24 48 72 and 96 hours after infestation A second infestation was carried out under initial conditions at 22 days after planting (DAP) and a third infestation at 26 DAP

Comparing the Imadicloprid Foliar Formulation Confidor 350 was applied similar to previous experiments using the same number of pots plants and insects per pot For each treatment seed was pre germinated in 3 g bags by placing it in water for 24 hours Treatments were as follows pre germination time of 24 36 and 48 hours insecticide dose of 6 12 and 24 ml/liter water and seed immersion time in insecticide solution for 15 30 and 60 minutes

Results and Discussion

Comparing pesticide treatment on dry and pregerminated seed When dry seed was immersed in fipronil solution for 60 minutes the control of adult *T orizicolus* reached 88% at 72 hours after infestation with insects as compared with 35% for seed immersed for 15 minutes At 144 hours control surpassed 90% in seed immersed for both the 30 and 60 minutes treatments (Figure 1) The trend of increased efficacy suggests that the seeds absorbed more pesticide as the treatment time was increased From a practical standpoint the 30 and 60 minute treatments were both acceptable

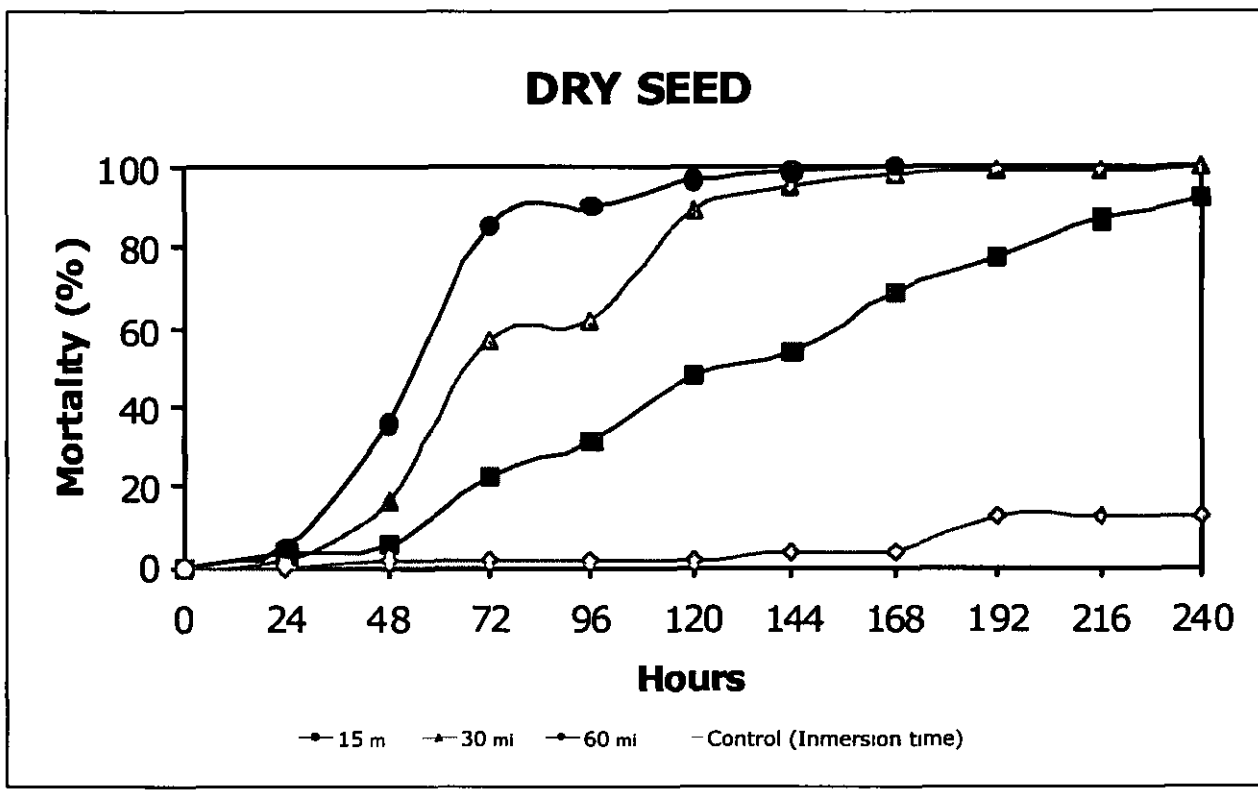


Figure 1 Controlling *Tagosodes orizicolus* by Applying Fipronil to Dry Seed

Compared to the dry seed treatment the percentage control of *T orizicolus* was increased by pregerminating seed for 24 hours probably because the seed absorbs both water and the insecticide Insect control is therefore enhanced as illustrated by improved control When pregerminated seed was immersed for 15 minutes there was more than 80% at 144 hours after inoculation with insects compared with only 50% control for the dry seeds

(Figure 2) When the seed was pregermination of seeds for 48 hours the results were similar to those when seed was immersed for 24 hours in insecticide solution reaching 100% at 120 hours after infestation with adult *T orizicolus* (Figure 3) After 192 hours all treatments presented 100% control of the insect as compared with 1 4% for the untreated check treatment

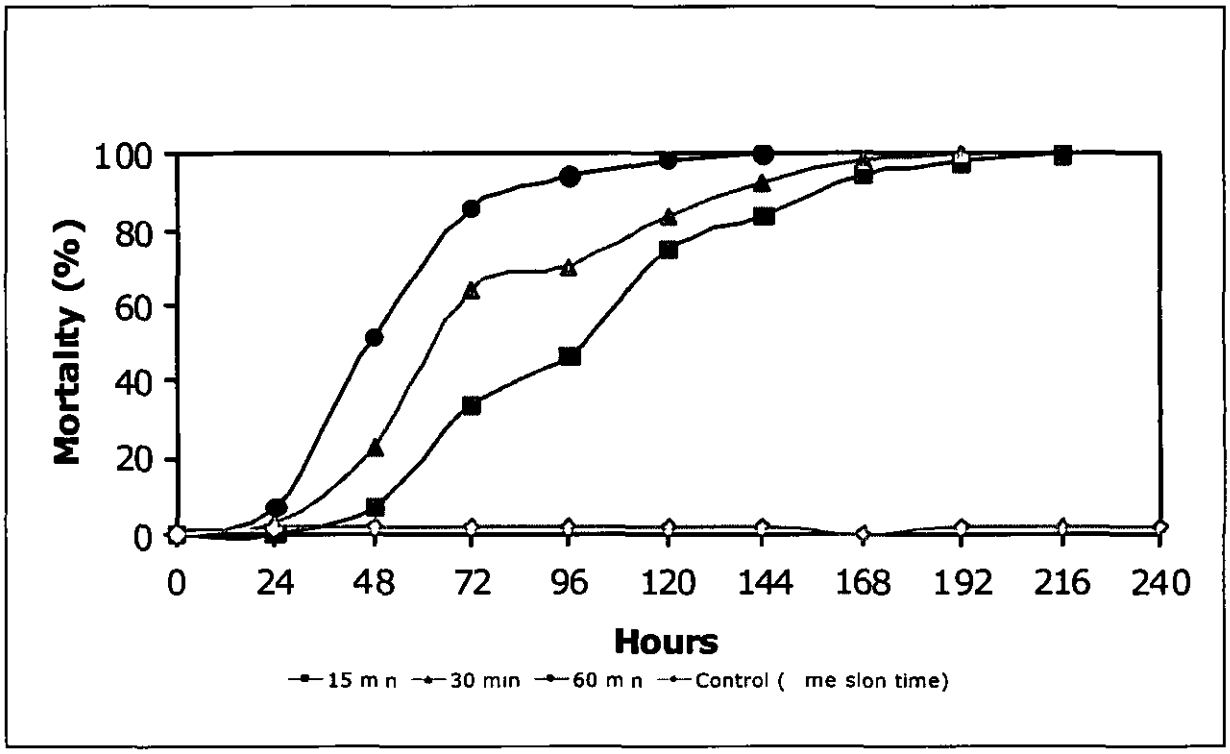


Figure 2 Controlling *Tagosodes orizicolus* by Applying Fipronil to Seeds Pregerminated for 24 hours

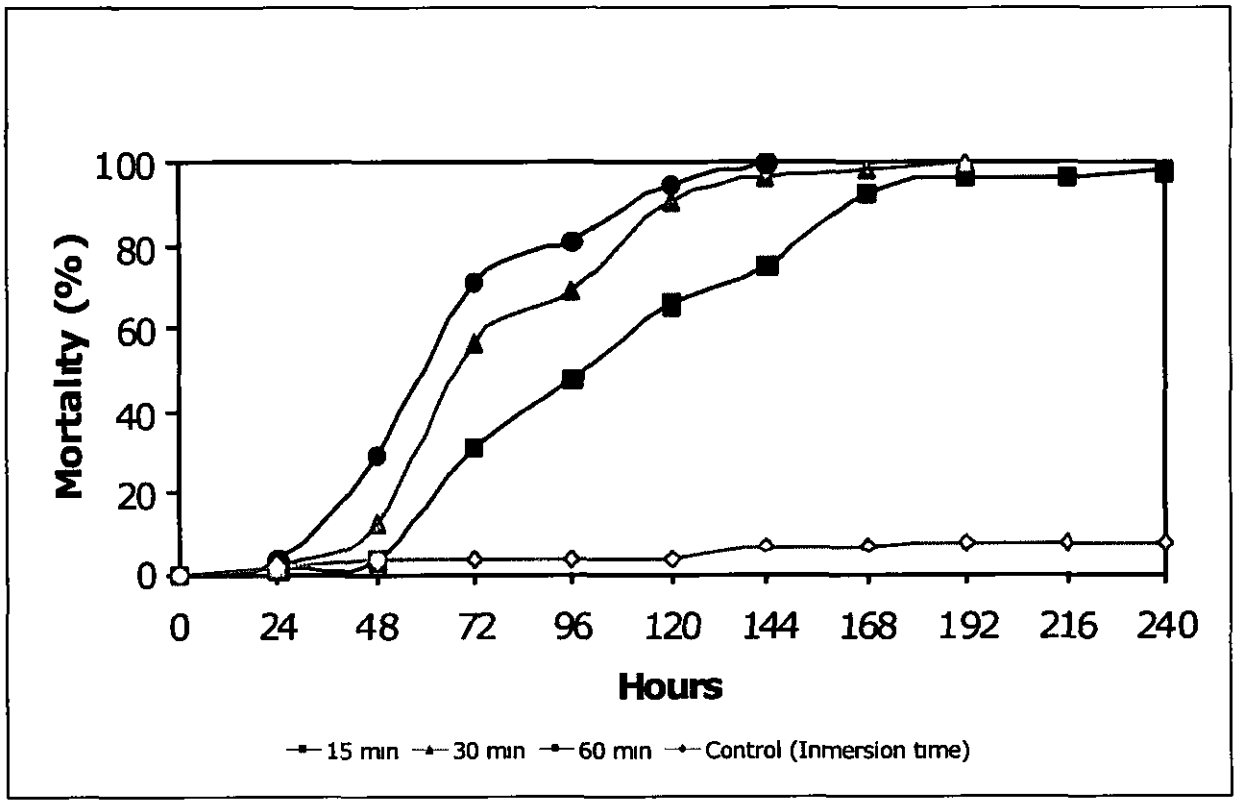


Figure 3 Controlling *Tagosodes orizicolus* by Applying Fipronil to Seed Pregerminated for 48 hours

Comparing the Efficacy of Fipronil and Imadicloprid At 24 hours after release of insects control of fipronil reached 91 5% compared with that of imidacloprid in a formulation for seed treatment (Gaucho) which only reached 11 0% at 15 days after planting (DAP) At 48 hours fipronil showed 100% control surpassing imidacloprid by 59 4% (Table 5) This same trend was maintained when insects were inoculated at 22 and 26 DAP Control of *T orizicolus* by fipronil surpassed that of Gaucho by 64% and 44% respectively at 48 hours after insect release

Table 5 Effects of the Insecticides Fipronil and Gaucho on Mortality of *Tagosodes orizicolus*

Time after insect release (hours)	Average percent of mortality of *Tagosodes orizicolus*					
	15 DAP[1]		22 DAP		26 DAP	
	Fipronil	Gaucho	Fipronil	Gaucho	Fipronil	Gaucho
24	91 5	11 1	51 0	17 0	74 0	31 0
48	100 0	40 6	93 0	29 0	97 0	53 0
72	100 0	87 3	100 0	57 0	100 0	90 0
96	100 0	95 0	100 0	70 0	100 0	93 0
120	100 0	95 0	100 0	98 0	100 0	100 0

1 DAP = Days after planting

Comparing the Imadicloprid Foliar Formulation Infestation of adult *T orizicolus* was carried out on the same day for all treatments and insect mortality was assessed at 5 days after infestation (DAI) After the first evaluation two re infestations were performed at 1 week intervals using the same amount of insects

Table 6 Effect of Confidor 350 for the Control of *T orizicolus* on Pre-germinated Rice Seed

Dose (ml/l)	Contact Time (minutes)	Average Percent Mortality of *Tagosodes orizicolus*		
		Hours of Pregermination		
		24	48	72
6	15	89 0	87 0	60 0
	30	92 0	73 0	71 0
	60	91 0	69 0	85 0
12	15	97 0	92 0	94 0
	30	98 0	96 0	90 0
	60	99 0	83 0	89 0
24	15	100 0	97 0	92 0
	30	100 0	71 0	95 0
	60	100 0	90 0	98 0

The effect of applying Confidor 350 on mortality of *T orizicolus* in each treatment is presented in Table 6 The dose of 24 ml/l of product was the most effective with 100% mortality The time seed was in contact with the insecticide did not affect mortality efficiency being similar with 15 30 or 60 minutes

When seeds were pregerminated for 24 hours and then immersed in 12 ml and 24 ml of Confidor 350 for 15 minutes the control of adult *T orizicolus* was 98% and 99% These control values decreased with pregermination time of seeds (Table 7)

Table 7 Effect of Confidor 350 for Controlling *T orizicolus* on Rice Plants at 26 Days After Planting

Dose (ml/l)	Contact Time (minutes)	Average Percent Mortality of *Tagosodes orizicolus* Hours of Pre-germination		
		24	48	72
6	15	58 0	59 0	58 0
	30	84 0	54 0	57 0
	60	68 0	43 0	80 0
12	15	98 0	63 0	88 0
	30	95 0	74 0	92 0
	60	97 0	60 0	91 0
24	15	99 0	81 0	90 0
	30	99 0	66 0	94 0
	60	98 0	77 0	100 0

Table 8 Effect of Confidor 350 for Controlling *T orizicolus* on Rice Plants at 38 Days After Planting

Dose (ml/l)	Contact time (minutes)	Average percent mortality of *Tagosodes orizicolus* Hours of pre-germination		
		24	48	72
6	15	70 0	24 0	37 0
	30	64 0	21 0	46 0
	60	62 0	16 0	53 0
12	15	97 0	52 0	74 0
	30	88 0	41 0	72 0
	60	79 0	43 0	78 0
24	15	96 0	37 0	78 0
	30	93 0	26 0	87 0
	60	96 0	45 0	92 0

Even at 38 DAP Confidor 350 continues to effectively control adult *T orizicolus* reaching 96% control with seed immersed in the insecticide solution for 15 minutes at doses of 24 ml/liter water

Conclusions

Control of *T oriziclous* with fipronil is quicker with seed pre germinated for 24 hours and then immersed in insecticide solution for 30 to 60 minutes Confidor 350 applied at 24 ml/liter water to seed pregerminated for 24 hours and in contact with the insecticide solution for 15 minutes is sufficient to obtain over 95% control of the insect in rice crops during the first 30 days after plant emergence When fipronil and Gaucho (imidacloprid) are compared fipronil controls more adult *T orizicolus* quicker surpassing Gaucho by more than 50% at 24 hours after insect release Seed treatments are cost effective methods to control pest during the first thirty days after planting The pregerminated seed at 24 hours appears to absorb the most pesticide Since the effect of the insecticide is mainly systemic this method should have the greatest impact on the insects feeding directly on rice and useful in integrated pest management of rice

OUTPUT 2 CHARACTERIZING RICE PESTS AND THE GENETICS OF RESISTANCE

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

Abstract

Few genes control the resistance to the RHBV and there are no known sources of complete immunity To ensure stable and durable resistance additional sources need to be identified and incorporated into rice Transgenic plants using the nucleoprotein (N gene) mediated cross protection have been developed and are showing stable RHBV resistance The N transgene could be used to complement the natural resistance source to the virus Besides RHBV the fungus *R Solani* causing sheath blight is increasing in importance in Latin America All commercial varieties are susceptible to varying degrees and no sources of complete resistance to this fungus are known Control depends on heavy applications of fungicides Incorporation of resistance for this disease by genetic engineering is an alternative approach Work conducted in collaboration with Rutgers University (USA) and IDEA (Venezuela) is focused on the use of a pokeweed antiviral protein (PAP) which has a ribosome inactivating ability with a potent antiviral and anti fungal activity against many plant viruses Mutated versions of PAP gene also confer resistance to the fungal pathogen *R Solani* among other fungal pathogens These results suggest the possibility of designing molecular strategies for incorporating dual antiviral and fungal resistance by introgressing mutant PAP gene(s) in transgenic rice plants We are reporting the progress made in the last year

- **Control of RHBV (Rice Hoja Blanca Virus) Through Nucleoprotein Mediated Cross Protection and in Transgenic Rice**

L Fory (SB2) E Tabares (SB2) A Mora (IP4) G Delgado (IP4) T Agrono (IP4) C Ordoñez (IP4) C Dorado (SB2) M C Duque (SB2 IP4) J Silva (IP4) L Calvert (IP4) Z Lentini (SB2 IP4)

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 Rice transformed with the RHBV nucleocapsid protein (*N*) gene had a significant reduction in disease development Reactions to inoculation with RHBV ranged from susceptible to completely resistant plants (immunity) The most frequent reaction was characterized by local necrotic lesions (hypersensitive reaction)

followed by the production of new leaves without symptoms Other plants developed chlorotic lesions in the inoculated leaves but recovered producing tillers free of virus (recovery phenotype) These transgenic RHBV resistant rice lines expressed the *N* gene RNA at low levels that could be detected using RT PRC but not by Northern blots analysis The nucleocapsid protein could not be detected in any of the transgenic plants either by Western or ELISA tests These results suggest that resistance conferred by the *N* gene is RNA mediated Earlier reports indicated that besides the resistant phenotype when challenged with RHBV the resistant transgenic lines showed significant increased performance for important agronomic traits including number of tillers number of grains per plant and yield as compared to the susceptible control Upon inoculation some of the resistant transgenic plants showed agronomic traits similar to the not inoculated Cica 8 control Using both agronomic traits and disease severity as criteria, several of the most resistant lines were followed through the R4 generation in the greenhouse and demonstrated that the *N* gene and RHBV resistance was inherited in a stable manner Results showed that the non transgenic F_1 control plants were susceptible whereas the transgenic F_1 plants were resistant even when inoculated 10 days after emergence These results suggested that the protection conferred by the RHBV N transgene is inherited and expresses independently of the genotype background The transgenic resistance could be used to complement the natural resistance source to the virus when crossing selected transgenic lines with diverse genotypes carrying the conventional resistance gene(s) This year report includes the characterization of mode expression of the transgenic resistance conferred by RHBV N The evaluation in the field for two semesters of transgenic lines representing various generations and F_2 populations derived from crosses with Fedearroz 50 Oryzyca 1 Iniap 12 and Cica 8 Planting was conducted at CIAT headquarters after receiving approval from the Colombian Biosafety Committee on September 2000 We also reported on the progress in generating transgenic rice containing the RHBV non structural 4 (NS_4) gene from the RNA 4

Materials and Methods

RHBV Resistance Assays

To characterize the effect of the plant age on RHBV N resistance plants of 15 or 28 days old after emergence (DAE) were inoculated with RHBV using four 2nd or 3rd instar *T oryzicolus* nymphs per plant from a colony with a virulence of 80% After 5 days of feeding on the test plants the vectors were killed using an insecticide application Controls consisted of transgenic plants of Cica 8 carrying only the hygromycin resistance (*hmr*) marker gene that was used in the selection process or non transgenic plants of Cica 8 Plants were scored for the development of RHBV disease symptoms every 3 days for 25 days and then evaluated once a week for 5 weeks Plants were scored for the date of the first appearance of symptoms and the percentage of leaf area affected by RHBV

For the field evaluations 280 transgenic lines and F_2 plants derived from crosses between resistant transgenic plants with Fedearroz 50 Oryzica 1 Iniap 12 and Cica 8 were planted in November 2000 and 486 transgenic lines and F_3 plants derived from the same crosses described

above were planted in July 2001 Lines were planted in a randomized plot design with 3 or 4 replications in the field for years 2000 and 2001 respectively Plants were inoculated at 15 days of age with an estimated two insects per plant from a colony of 80% virulence Insects were allowed to feed on the plants for 15 days in the first season and for 10 days in the second season when insecticide was applied as a biosafety control measurement Plants were evaluated for the development of disease symptoms every two weeks until 45 days of age Disease evaluations were conducted using an scale from 0 to 9 were 0 refers to no disease symptoms and 9 indicates more than 90% leaf area is affected by the RHBV disease Plants with rating from 1 to 3 = resistant score 5 = intermediate and 7 9 = susceptible

Results and Discussion

- **Comparative Level of RHBV N Resistance in Transgenic Rice in the Greenhouse Respect to the Field**

L Fory (SB2) T Agrono (IP4) M Cruz (IP4)[1] C Ordonez (IP4) G Delgado (IP4) C Dorado (SB2) M C Duque (SB2 IP4) J Silva (IP4) L Calvert (IP4) Z Lentini (SB2 IP4)

Last year we showed that line A3 49 60 12 3 3 showed the highest level of resistance throughout the whole life cycle Between 74% to 81% of the plants did not show any disease symptoms when inoculated either at 15 days or 28 days of age and only a 22% of the plants showed more than 25% of the leaf area affected when inoculated at 15 DAE (Table 1) In contrast Cica 8 control showed 100% of the plants with severe disease symptoms at the15 DAE inoculation (Table 1) Line A3 49 60 4 5 8 showed intermediate level of resistance at the 15 DAE inoculation (Table 1) and 71% of the plants did not have symptoms at 28 DAE inoculation About 70% of the plants of line A3 49 60 19 had less than 25% of leaf area affected at 15 DAE inoculation (Table 1) Sister lines A3 49 60 12 3 1 (susceptible) y A3 49 60 12 3 3 (resistant) showed different disease reaction indicating that the resistant phenotype was still segregating at the T_4 generation or gene silencing was affecting the expression of the RHBV N gene in some of the plants This year results indicated that there is a high correlation between level of resistance seen in the greenhouse with that in the field (Table 1)

[1] Currently with FLAR

Table 1 Comparative Disease Reaction in the Greenhouse and the Field in Plants Inoculated at 15 days after emergence

	Greenhouse Leaf Area Affected (/)			Field[3] Disease Reaction	
Line	0	>0 25	>25-100	2000	2001
A3-49 60 12 3 3	74	4	22	1	2
A3-49 60 19	53	12	36	2	3
A3-49 60-4 5 8	54	0	46	3	4
A3-49 60 13	25	4	70	3	4
A3-49 56-15	9	13	78	6	6
A3-49 60 12 3 1	7	0	93	6	7
A3-49 101 18 19 2	15	0	85	8	6
A3-49 78[2]	0	0	100	ND	7
Cica 8	0	0	100	9	7

[2] Transgenic control with the *hmr* gene only ND = not determined
[3]Mean values of three (in 2000) or four (in 2001) replicates

- **Field Evaluation of RHBV Resistance in Transgenic Rice Containing the Nucleoprotein Gene**

Mora, A Fory L Tabares E Lozano I Agrono T Cruz M Ordonez C Delgado G Bolanos E Rios A Tigreros H Duque M Silva, J Calvert L Lentini Z

Advanced generations of transgenic lines with stable RHBV resistance were selected in the greenhouse until the permit for the field test was granted by the Colombian Biosafety Committee on September 2000 The first field trial to conduct evaluations for RHBV resistance and agronomic traits following the Colombian environmental biosafety regulations was planted on November 2000 at the biosafety rice field located in CIAT experimental station Palmira (PES)

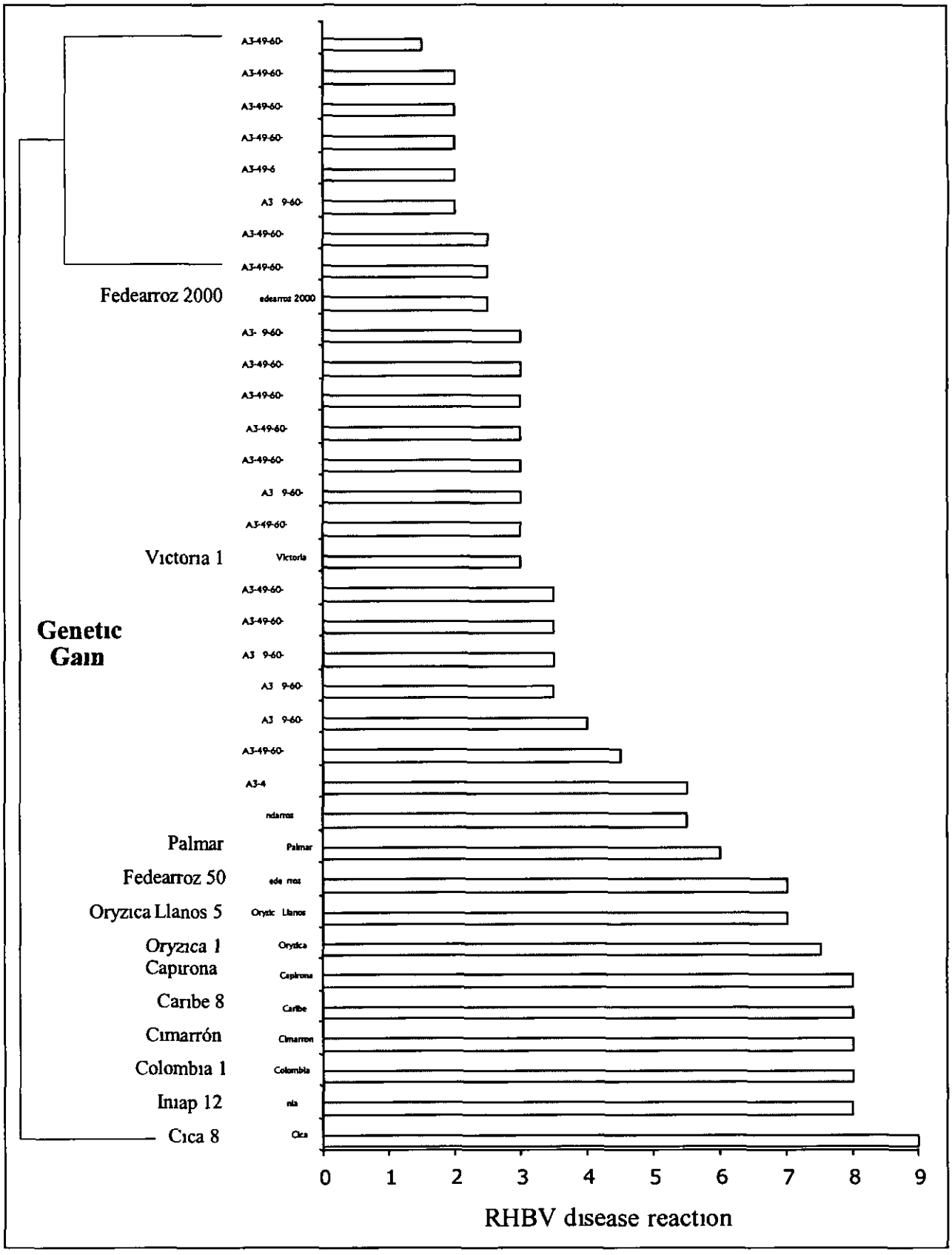
Replicated trials were conducted as described in materials and methods Disease evaluations were conducted using an scale from 0 to 9 were 0 refers to no disease symptoms and 9 indicates more than 90% leaf area is affected by the RHBV disease Plants with rating from 1 to 3 = resistant score 5 = intermediate and 7 9 = susceptible The variety Cica 8 and the transgenic Cica 8 line (A3 49 78) carrying only the hygromycin resistance (*hmr*)gene which was used as the selectable marker to generate the transgenic plants were used as controls Other varieties were used as reference for differential disease reaction pattern These differential varieties included Caribe 8 Capirona Cimarron Colombia 1 Fedearroz 50 Fedearroz 2000 Fedearroz Victoria 1 Fundarroz PN1 Iniap 12 Linea 2 Oryzica 1 Oryzica Llanos 5 and Palmar

Field evaluations corroborated results obtained previously in the greenhouse Forty five entries derived from line A3 49 60 12 3 3 were highly resistant showing scores 1 to 3 A subset of these lines are shown in Table 1 Resistance in advanced transgenic lines (T_6 and T_7) was inherited in a stable manner (95% of derived progeny plants were reproducibly resistant) indicating that the RHBV N transgene was fixed in each of these transgenic lines (Table 1) The most resistant lines were A3 49 60 12 3 3 79 A3 49 60 12 3 3 24 A3 49 60 12 3 3 28 A3 49 60 12 3 3 32 A3 49 60 12 3 3 68 A3 49 60 12 3 3 67 A3 49 60 12 3 3 72 (Figure 1) These lines were more resistant than Fedearroz 2000 on average over the two field evaluations (Figure 1) Other transgenic lines were intermediate for resistance giving ratings between 3 to 5 but several transgenic lines were as resistant as Fedearroz Victoria I (Figure 1) A large number of the lines were more resistant than the commercial varieties and the resistance genetic gain from Cica 8 to the transgenic lines was a reaction score change from 7 9 (Cica 8) to 1 3 (best transgenic lines) (Figure 1) The transgenic line A3 49 78 which only carries the hygromycin resistant gene but not the RHBV N gene was as susceptible as Cica 8 (Table 2) indicating that the resistance noted in the RHBV N transgenic lines was due to the RHBV *N* viral gene Fedearroz 50 the main variety currently commercially grown in Colombia was rated as susceptible under the conditions of high disease pressure in this trial (score 7) and it was more susceptible than Fundarroz PN1 (score 5 5) which reacted as intermediate or Fedearroz 2000 that rated as resistant (Figure 1) Similar progress towards resistance was noted in the crosses between the highest resistant transgenic lines and the varieties Crosses between non transgenic Cica 8 and Fedearroz 50 Oryzica 1 and Iniap 12 gave an average disease reaction of 8 0 7 0 and 8 5 respectively corresponding to susceptible phenotype in contrast the corresponding crosses generated with the transgenic resistant plants and the same varieties showed an average disease reaction of 4 5 4 0 and 4 0 respectively The crosses are at F_3 generation thus the RHBV N gene is still segregating in those lines Resistant plants (score 2 3) within those segregating F_3 lines had been identified These field results corroborate earlier findings indicating that the protection conferred by the RHBV N transgene is inherited and expressed independently of the genotype background and that the transgene can be transferred to breeding populations by standard crossing Sister lines of the resistant transgenic lines and transgenic crosses evaluated last season indicated that some of the plants showed a yield potential similar to varieties as Fedearroz 50 Fedearroz Victoria 1 and Oryzica 1 Progeny plants derived from resistant plants selected in the first field evaluation are now being selected for yield potential and other agronomic traits

Table 2 RHBV Resistance Evaluations of Trasngenic RHBV N Plants Over Two Consecutive Seasons in the Field

Line	Score[1]	
	2000	2001
A3-49 60 12 3 3 68	1	3
A3-49 60 12 3 3 32	1	3
A3-49 60 12 3 3 79	1	2
Fedearroz 2000	2	3
Victoria 1	2	4
A3-49 60-12 3 3 24	2	2
A3-49 60 12 3 3 28	2	3
A3-49 60-4 5 8 79	2	4
A3-49 60 12 3 3 72	2	3
A3-49 60 13 2	2	2
A3-49 60 12 3 3 19	3	3
A3-49 60 12 3 3 18	3	4
A3-49 60 12 3 3 78	3	3
A3-49 60 13 8	3	4
A3-49 60 13 1	3	3
A3-49 60 12 3 3 77	3	4
A3-49 60 12 3 3 12	3	3
A3-49 60 12 3 3 59	3	4
A3-49 60 12 3 3 3	3	3
A3-49 60 12 3 3 14	3	3
A3-49 60 12 3 3 67	3	2
A3-49 60 19 8	4	4
A3-49 60 12 3 3 23	5	4
A3-49 101 18 19 2	5	6
Fundarroz PN1	5	6
Palmar	6	6
Iniap 12	8	8
Colombia 1	9	7
Oryzica 1	8	7
Capirona	8	7
Caribe 8	8	8
Cimarron	8	8
Fedearroz 50	7	7
Linea 2	6	7
Oryzica 1	7	7
Oryzica Llanos 5	7	7
A3-49 60 10 27	ND	1
A3-49 78 *	ND	7
Cica 8	9	7

( ) Transgenic line carrying only hygromycin resistance gene It does not contain the RHBV N transgene
[1]Mean values of three (in 2000) or four (in 2001) replicates


Genetic Gain
Fedearroz 2000
Victoria 1
Palmar
Fedearroz 50
Oryzica Llanos 5
Oryzica 1
Capirona
Caribe 8
Cimarrón
Colombia 1
Iniap 12
Cica 8
0
1
2
3
4
5
6
7
8
9
RHBV disease reaction

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

L Fory (SB2) E Tabares (SB2) I Lozano (IP4) G Delgado (IP4) T Agrono (IP4) C Ordonez (IP4) C Dorado (SB2) M C Duque (SB2 IP4) J Silva (IP4) Z Lentini (SB2

Introduction

The genome of RHBV consists of four species of ssRNA designated RNA 1 2 3 and 4 The RNA 4 consists of 1991 nucleotides with two open reading frames (ORFs) The RNA 4 encodes a major non structural protein (NS_4) which accumulates in the tissues of the infected plants with the RHBV NS_4 protein is clearly distinguishable from the nucleoprotein (N) by specific antiserum Another difference is that the NS_4 protein is only expressed in the plant while the N protein is expressed both in the plant and the insect vector It is inferred from the differential expression of these proteins that the major NS_4 protein may have a function that is needed in the plant but not in the plant hopper The NS_4 protein does have some similarity with the helper proteins of Cauliflower mosaic virus which suggests that NS_4 might be involved in the transmission of RHBV from the plant to the plant hopper 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 to study the potential for a novel and different method of producing viral resistant plants

Materials and Methods

Rice Transformation

Mature embryos derived calli from *indica* varieties CICA 8 Palmar Cimarrón and Fundarroz PN1 where used as targets We used *Agrobacterium* Agl1 strain mediated transformation to introduce the NS_4 gene Constructs *pIC002* and *pIC004* contained the RHBV NS_4 gene in sense and anti sense orientations respectively driven by the 35S CaMV promoter using the plasmid *pCAMBIA* 1301 which carries the *gus intron* and hygromycin resistance genes Constructs *pIC007*and *pIC009* contain the NS_4 sense gene and *pIC008 the NS_4* anti sense gene driven by the ubiquitin or 35 S CaMV promoter These genes were cloned into *pWBVec8* plasmid (from Peter Waterhouse s laboratory at CSIRO Australia) which carries the hygromycin cat 1 intron gene as selectable marker Plants were regenerated after stepwise selection on 30 mg/l and 50 mg/l hygromycin followed by 50 mg/l hygromycin throughout plant differentiation Plants were grown to maturity in the biosafety glasshouse

Molecular Analysis of the Transgenic Rice Plants

Southern Analysis Fifteen µg DNA genomic were digested with the restriction enzyme Eco RI fractionated in 1 0 % (W/V) agarose gels and transferred to nylon membranes (N+ Amersham) The hybridization probe was a radioactively labeled 850 base pair PCR fragment amplified using primers specific for RHBV *NS4* gene DNA probes were labeled using random primers and the hybridization was carried out overnight at 55 C

RT PCR and Northern Analysis Total RNA was extracted from 100 mg of fresh material using the RNAeasy ™ plant total RNA kit (Quiagen Dorking UK) The cDNA synthesis was done with the SUPERSCRIPT™ One Step RT PCR system following the manufacturer s instructions and using RHBV NS 4 forward and reverse primers Northern analysis was carried out using 15 µg of total RNA per lane in denaturing formaldehyde and formamide agarose gels (Sambrook et al 1989)

Characterization of Sequence of NS4 Gene Nucleotide sequence of the gene NS_4 in four T_0 generation transformed plants was carried out using the ABI PRISM Dye terminator kit (Perkin Elmer) with RHBV NS 4 forward and reverse primers These PCR products were directly sequenced using a Biosystems Prism 377 DNA sequencer (Perkin Elmer) and edited with Sequencher (Genecodes Ann Arbor MI) The sequences were analyzed using the BLAST algorithm (Altschul et al 1997)

RHBV resistance assays Inoculations and evaluations were conducted in the greenhouse following the same procedure as described above for the RHBV N transgenic plants

Results and Discussion

Last year we reported the generation of 10 different constructs carrying the NS_4 gene in sense and anti sense orientations A total of 21 transgenic plants carrying the NS_4 sense orientation and 70 plants carrying NS_4 anti sense orientation were produced (Table 1) The plant regeneration efficiency varied according to the genotype from 2% to 44% Southern blot analyses using the *Bam HI* or *Eco RI* which excise the complete NS_4 gene in either sense or antisense orientation indicated that between 50% to 100% of the regenerated plants analyzed contained the NS_4 gene (Table 1) Further analysis using *Sal I* which does not cut the gene cassette within the right and left borders indicates that in most cases the NS_4 gene is integrated as a single non rearranged copy

Table 1 Transformation Efficiency of Three *indica* Varieties Using the RHBV NS_4 in Sense an Anti Sense Genes

Genotype	Plasmid	S_4	Plants	RE /	Plants S	/ Plants S	TE /
PALMAR	pICOO7	Sense	1	2	ND		
	pICOO9	Sense	1	3	ND		
	pICOO4	@Sense	13	28	13/13	100	28
	pICOO8	@Sense	9	20	6/6	100	20
CICA 8	pICOO2	Sense	14	33	11/14	79	26
	pICOO7	Sense	6	12	2/3	67	8
	pICOO9	Sense	18	44	18/18	100	18
	pICOO8	@Sense	10	18	5/10	50	9
CIMARRON	pICOO8	@Sense	19	48	ND	ND	ND

@Sense = anti sense RE = plant regeneration efficiency S = Southern positive TE = transformation efficiency

The NS_4 gene was also amplified by PCR generating a product of the expected gene size The PCR product was sequenced and in all cases the sequence corresponded to the entire NS_4 gene indicating that the transgene was not rearranged PCR analysis for the *gus intron hmr* and *NS_4* transgene and Southern blot RT PCR and Northern analyses for the NS_4 of T_1 plants derived from T_0 identified as transgenic by Southern blot indicated that not all T_1 plants inherited the three transgenes (i e *gus intron hmr* and *NS_4*)(Table 2) Variation in the level of *gus* expression was also noted and the expression of either *gus* or NS_4 was not always detected even though plants contained the corresponding gene These results suggest that gene silencing was occurring in some of the transgenic plants (Table 2) Most plants showed low levels of RNA expression from either the NS_4 sense or anti sense genes In these plants the RNA was detected by RT PCR NS_4 gene expression was detected by regular Northern in only one plant so far (Table 2)

Table 2 Gus intron hmr, and NS_4 transgenes inheritance and NS_4 expression in T_1 transgenic plants

		Plant	Plant	PCR[2]					NS_4	
Genotype	Plasmid	T_0	T_1	GUS[1]	*gus*	*hmr*	*NS_4*	S[3]	RT PCR	Northern
Cica 8	pIC002	1	1						ND	ND
		1	2							
		1	5	+					ND	ND
		2	11						ND	ND
		7	1	+++	+	+	+		ND	ND
		7	2	+++	+	+	+		ND	ND
		7	3	+++	+	+	+	+	ND	ND
		7	4		+	+	+	+		
		7	18	++	+	+	+	+	+	
		9	14	+++	+	+	+	+		
		9	15	+++	+	+			ND	ND
		12	7	+++	+	+	+	+		+
		12	11	++	+	+			ND	ND
		12	15	+++	+	+			ND	ND
Cica 8	None	NT	NT							
Palmar	pIC004	1	18	+						
		1	24							
		10	16	++	+	+			+	
		4	3	+++	+	+	+	+	+	
		4	5	+++	+	+	+		+	
		4	17	+++	+	+	+	+	+	
		4	18							
		4	20	+++	+	+	+	+	+	
		4	25	+++	+	+	+	+	+	
		7	4		+	+				
		7	16		+	+	+		+	
		7	22	+	+	+			+	
		7	23						ND	ND
Palmar	none	NT	NT							

[1] Test of Gus in leaves The expression of GUS gene was scored based on the level of expression (+) low (++) intermidate (+++) high [2] PCR analysis to detect the transgenes *gus* *hmr* hygromycin *NS_4* [3] S = + Positive to Southern blot analysis ND= Not determined T_1 plants derived from the same T_0 plant are sister lines NT = Not transgenic

T_1 plants derived from self crosses of eight T_0 plants originally identified as transgenic based on Southern analysis were selected to conduct the RHBV resistance evaluations in the greenhouse Each T_0 line was represented by 13 T_1 plants and were inoculated at 18 days after germination with four insect vectors per plant derived from a colony with 80% virulence Parallel to the inoculations 25 T_1 sister plants were used to determine the level of GUS expression Results indicated that most T_1 plants from line 4 transformed with plasmid pIC004 showed the expected GUS expression indicating inheritance of the gus intron gene In this line the number of plants with no or minor disease symptoms (<10% leaf area affected) was double respect to the non transgenic control (Table 3) No differences in disease reaction were noted for the other transgenic lines and the corresponding control (Table 3) However for these lines there is indication that the transgene inheritance is significantly deviating from a Mendelian segregation and in 5 out of the 8 lines evaluated showed less than 25% T_1 plants with the transgene in comparison to the 75% expected Because of this skewed segregation which is commonly found in early generation of transgenic plants it is necessary to advanced to the T_2 generation from those T_1 transgenic plants carrying and expressing the corresponding transgenes The T_2 plants that inherit and express the NS_4 gene will need to be identified by molecular analyses and further resistance studies will be made

Table 3 Disease Resistance on T_1 Transgenic Plants Derived from Different T_0 Plants Carrying NS_4 Sense (pIC002) or NS_4 Anti Sense (pIC004) and Inoculated with RHBV at 18 Day Old in the Glasshouse

Genotype	Plasmid	T_0 Plant	/ plants Gus[1]	Leaf Area Affected (/ Plants)	
				≤ 10	>10 100
Cica 8	pIC002	1	4	0	100
		7	4	0	100
		9	40	15	85
		12	40	0	100
	None	Control	0	0	100
Palmar	pIC004	1	12	31	69
		4	84	62	38
		7	12	46	54
		10	24	46	54
	None	control	0	31	70

Eighteen plants were evaluated per each T_1 line [1] 25 plants per T_1 line were tested for GUS expression

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Foreign Genes as Novel Sources of Resistance for Fungal Resistance

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Introduction

The fungi *Rhizoctonia solani* (sheath blight) *Helmithosporium Rhincosporium* and *Sarocladium* cause important rice yield losses in the Southern cone of South America and they are become more prevalent Colombia, Mexico and Venezuela Although there is some degree of resistance most commercial rice varieties are susceptible and there are few known sources of stable genetic resistance for these diseases in rice In the case of sheath blight IRRI had placed a major effort in developing biological control strategies for this disease At present in Latin America, the control of this complex mainly depends on use of fungicides It was decided that the lack of good genetic resistance made it appropriate to develop molecular strategies for incorporating resistance to this fungal complex Of the four the plant pathogen interaction with *Rhizoctonia solani* is the better known

Work conducted by another principal investigator of this project (Dr Nilgun Tumer Biotechnology Center at Rutgers University USA) showed that a pokeweed antiviral protein (PAP) a 29 kDa protein isolated from *Phytolacca americana* (a weed naturally found from USA to Argentina) has a ribosome inactivating ability Mutated versions of PAP gene has potent antifungal activity (Zoubenko et al 1997) Homozygous progeny of transgenic tobacco plants expressing these PAP genes displayed resistance to the fungal pathogen *Rhizoctonia solani* Transgenic PAP potato showed protection against *Phytophtora infestans* and transgenic PAP turfgrass are resistant to various fungal pathogens These results suggest the possibility of designing molecular strategies for incorporating fungal resistance by introgression of mutant PAP gene(s) in transgenic rice plants Here we report the progress made during the second year of this project

Last year we reported that eight new PAP mutations were generated by site specific mutation of aminoacids in the PAP protein These new mutant genes were placed into yeast vectors and transformed into yeast to check for their toxicity The non toxic mutated genes are being transformed into tobacco to check the gene expression and toxicity before using them to transformation rice Two mutated versions of PAP (I deleted and II) which were found to be non toxic in turfgrass (another monocot species) were used as the first candidate PAP genes The expression of these genes is controlled by the ubiquitin promoter and they were kindly supplied by Dr Peter Waterhouse (CSIRO Australia) in the plasmid vectors pWBVec8 pWB10a, and pBGXiHGFP These plasmids had been used successfully in Dr Waterhouse laboratory to transform rice via *Agrobacterium* They contain a hpt gene with a CAT 1 intron for increased expression of hygromycin resistance and to aid in the selection of transformed plants they contain either a gus intron gene or a gfp (green fish fluorescent) gene A total of 35 independent

transgenic events carrying the PAPI deletion mutant gene and 50 independent transgenic events carrying the PAPII gene were generated last year A first set of plant tissue was sent to Rutgers this summer for analysis and plants with PAP gene expression were identified based on Western analysis This year a new version of the PAP gene (PAPY123) which include a deletion of 3 nucleotides was used to generate another set of transgenic plants

Materials and Methods

Transformation and Plant Regeneration

Mature embryos derived callus of *indica* varieties CICA 8 Palmar Cimarron and Fundarroz PN1 were used as targets Palmar and Cimarron show a high and moderate tolerance to sheath blight whereas Fundarroz PN1 and Cica 8 are highly susceptible to sheath blight. The transformation experiments were conducted using *Agrobacterium tumefaciens* strain Agl1 (Wang et al 1997) carrying one of the following plasmids NT305 NT306 and NT446 Maize ubiquitin or 35S CaMV promoter drives these plasmids which carry various mutant versions of the PAP gene (PAPI PAPII and PAPY123) The hygromycin resistance conferred by the *hpt cat intron gene* was used as the selective marker Plants were regenerated after stepwise selection on 30 mg/l and 50 mg/l followed by other 30 mg/l hygromycin selection throughout plant differentiation Plants were grown to maturity in biosafety glasshouses

Molecular Analysis of the Transgenic Rice Plants

Southern blot and PCR analyses were used to detect the presence of the PAP and hygromycin genes For this purpose 15 µg of DNA were digested with different restriction enzymes The gels were denatured and neutralized by standard procedures The DNA was transferred to nylon membranes (Hybond N Amersham) The filters were hybridized at 60°C The presence of the kanamycin gene was determined throughout PCR

Results and Discussion

A total of 59 transgenic plants carrying the PAPY123 gene were generated and confirmed by Southern blot (Table 1) More than 92% of the regenerated plants had integrated the PAPY123 gene in the genome as determined by Southern blot analysis (Table 1) A similar number of plants were generated for each of the PAP genes (Table 2)

This year to confirm the integration of the PAP gene 63 plants of the varieties Palmar and Cica 8 transformed with the plasmids NT 446 were evaluated by Southern blot and PCR methods The genomic DNA was digested with different restriction enzymes (Bam HI/EcoRI and Hind III) to excises the PAP gene The results indicated that at least one copy of the PAP gene was integrated

into the rice genome Moreover a better analysis on the patterns of integration was obtained using the enzymes BamHI/EcoRI that cut the PAPY123 gene into two fragments (Figure 1) This study showed that 67% of the plants analyzed had only one copy of the gene apparently without any rearrangements (Figure 1) The PCR analysis of some plants confirmed the presence of the PAPY123 gene At present the transformation of the PAPY123 gene has been accomplished for the varieties Palmar and Cica 8 The Southern blot analysis also revealed the presence of the hpt and nptII genes From Western blot analysis using PAP specific antisera about 50% of the plants analyzed expressed the PAPY123 protein whereas 18% of the plants tested were expressing either the PAPI or PAPII protein The transformed plants expressing the PAP protein will be evaluated for resistance to sheath blight under greenhouse conditions Also detailed molecular analyses will be conducted to determine the gene copy number of these plants

Table 1 Transformation Efficiency of Three *indica* Varieties Using the AgII (NT446)

Genotype	Plasmid	Callus	Plants	RE /	Plants Analyzed by Southern	Plants S	/ Plants S+	/ TE
PALMAR	NT446	182	48	26 4	36	34	94 4	25
CICA 8	NT446	220	34	15 4	27	25	92 5	14 2
CIMARRON	NT446	16	5	31 2	ND	ND	ND	ND

Five replicates One replicate RE = plant regeneration efficiency S = Southern positive TE = transformation efficiency

Table 2 Comparing of the Number and Time of Plant Regeneration Using Three Different Versions of Gen PAP

Genotype	Plasmid	Time of Regenerated plants (Months)	No Plants	/ Western Blot
PALMAR	NT446	4	39	
CIMARRON	NT446	3 5	5	
CICA 8	NT446	4	27	
Total 446			71	50
PALMAR	NT305	4 5	23	
CIMARRON	NT305	5 5	33	
CICA 8	NT305	5 5	6	
Total 305			62	18
PALMAR	NT306	5	33	
CIMARRON	NT306	5 5	14	
CICA 8	NT306	5 5	12	
Total 306			59	18

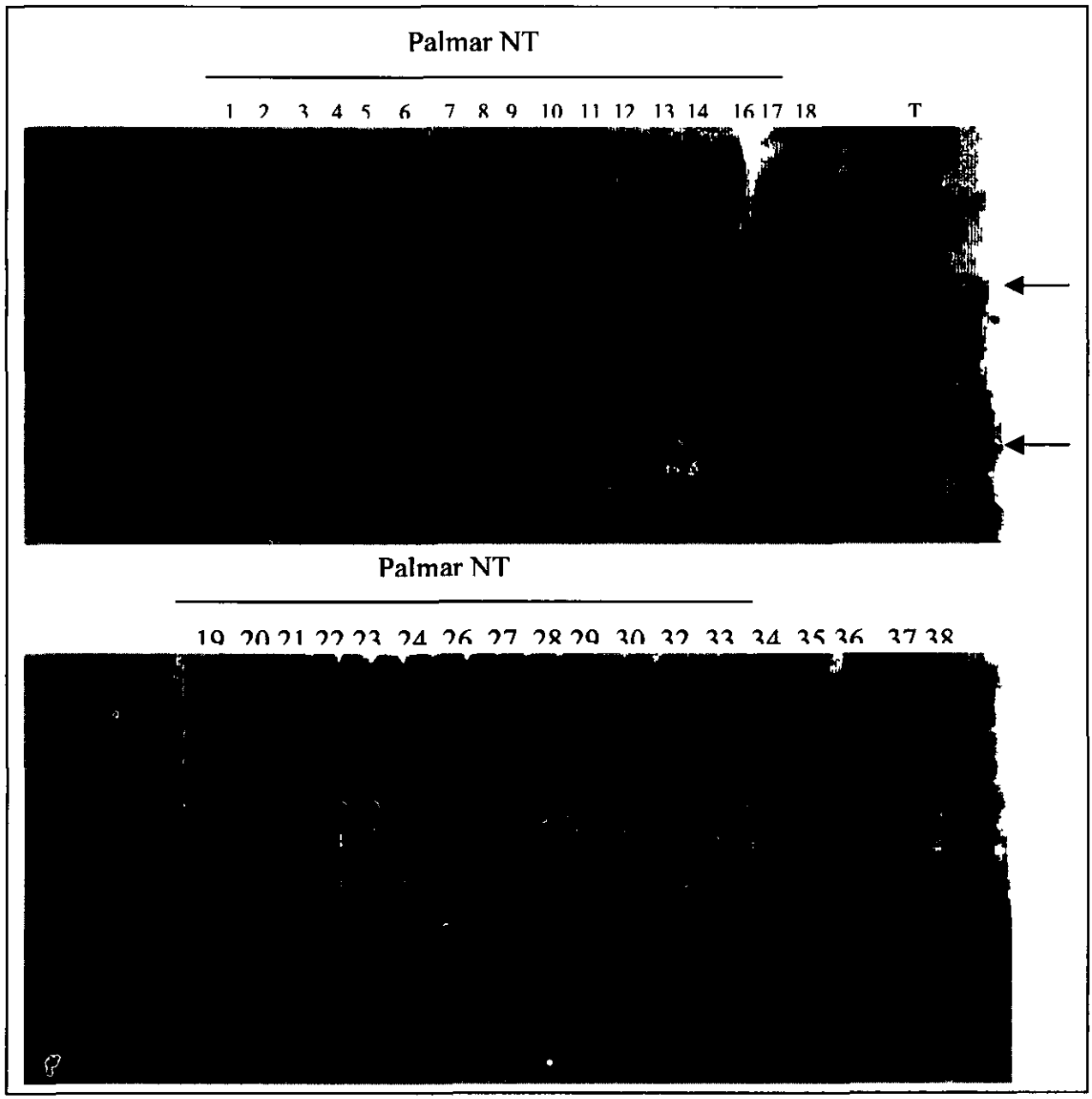

Figure 1 Southern blot analysis of genomic DNA of T_0 plants transformed with *PAP* Y123 gene DNA was cut with *Bam HI* and *Eco RI* T = Control non transgenic P = Plasmid NT446 carrying PAPY123

OUTPUT 2 CHARACTERIZING RICE PESTS AND THE GENETICS OF RESISTANCE

2E Characterization of Crinkling Disease A Complex of *Polymixa graminis* and Rice Stripe Necrosis Virus

L Calvert and R Sedano

Abstract

Greenhouse screening for resistance to crinkling disease was used to identify resistant germplasm from several sources The best source of resistance is *O glaberrima* but even some of the commercial varieties have a fair level of resistance to crinkling disease and should be used in breeding programs Several of the VIOFLAR/1999 lines were found to be highly resistant Transplanting rice was found to increase disease incidence and contaminated soil was a better source of inoculum than contaminated seeds A short seed heat treatment was found to be ineffective at reducing disease incidence Using soils contaminated with *P graminis* that contains RSNV was found to inhibit root growth Since the results could not be correlated with the incidence of RSNV further studies are needed to determine the effect of *P graminis* alone or if other soil biotic factors were affecting the root growth

Introduction

Crinkling disease was first reported in West Africa (Louvel and Bidaux 1977) A complex of rice stripe necrosis virus (RSNV) and *Polymyxa graminis* causes this disease (Fauquet and Thouvenel 1983 Fauquet et al 1988) A similar disease called Entorchamiento appeared in Colombia in 1991 and this is also caused by RSNV and *P graminis* (Morales et al 1999) This disease has spread rapidly throughout Colombia and is present in most of the important rice growing regions It is also found in very low incidence in Panama The resting spores of *P graminis* are transmitted on the seeds that are contaminated with soil and the dry resting spores are stable for many years Outbreaks of crinkling disease are sporadic and are not easy to predict The damage done by crinkling disease occurs in the first month after planting Many of the infected plants die others are severely stunted the leaves are malformed and some leaves have chlorotic stripes The roots of the infected plants can be stunted and often turn necrotic Since the disease is most severe when young plants are infested later infections do not appear to cause any additional losses In most infested fields there are a few patches with a high disease incidence while the incidence in the remainder is low

Materials and Methods

Screening Germplasm for Resistance to Crinkling Disease Rice was planted in shallow trays using contaminated soils obtained from fields with a high incidence of crinkling disease The soil was kept humid to allow the exposure of the germinating roots to the zoospores of the *P graminis* On average twelve days after seeding the rice was transplanted to pots (10 plants/pot) that contained a mixture of contaminated soil (50%) and sand (50%) Additionally the contaminated soil was inoculated with pulverized roots containing cystosori of the vector Materials were evaluated using at least three repetitions of ten plants The pots are maintained in Plexiglas trays and the plants are evaluated for symptoms after 35 days A total of 265 lines were evaluated for their reaction to crinkling disease These included 212 lines in the VIOFLAR germplasm bank 23 CT lines 17 commercial varieties 10 lines of *O glaberrima* and three of *O rufipogon*

Comparing the Effect of Direct Seeding and Transplanting on the Incidence of Crinkling Disease Oryzica 3 and Fedearroz 2000 FL00478 29P 18 1P M (line 105) and FL00518 1P 4 3P M (line 113) were used in this experiment and are susceptible to crinkling disease For half the treatments the seeds were mixed with soil from fields that had a high incidence of crinkling disease The soil and seeds were mixed together at a ratio of 10w/1w The seed mixture was dried for 12 hours at room temperature and the excess soil was removed The control seeds were treated in a similar manner using contaminated soil that was sterilized using two hours of steam treatments The contaminated seeds were either planted directly or transplanted at 12 days These treatments were compared with clean seeds that were either planted directly or transplanted into contaminated soils

Seed Treatments Heat treatments were made to evaluate their effect as a control measure for crinkling disease Groups of forty seeds of Oryzica 3 were mixed with contaminated soils After drying an removing excess soil the seedlots were exposed to dry heat of 26 55 65 75 and 85 C for 3 minutes The seeds were planted into sterile soil and the incidence of crinkling disease was evaluated at 30 days The experiment was repeated three times

Effect of Crinkling Disease on the Root Development of Rice Eleven rice lines were tested for their reaction to crinkling disease using root weight as the criteria Ten seeds per pot were planted in contaminated soil or sterile soil and the pots were placed in the greenhouse conditions in a randomized design There were three repetitions for each variety and treatment After the plants were evaluated at 35 days for the foliar symptoms of crinkling disease each pot was placed into a container of water and the soil was gently washed away The results were analyzed by ANOVA using SAS (SAS Institute Cary NC USA)

Results and Discussion

Screening Rice Germplasm for Resistance to Crinkling Disease The screening of the VIOFLAR/1999 collection was done to obtain an idea of the level of resistance that is in one set of breeding lines Selected *O glaberrima* lines were tested since they are known to be resistant to crinkle disease Also commercial varieties were tested to see if resistance is in the current varieties The majority of the lines tested were susceptible to crinkling disease and 89% of the lines had more than 40% incidence of the disease (Table 1) The *O glaberrima* lines proved to be highly resistant and no infections were found using the greenhouse assay (Table 2) The rice line that had the best resistance and has been tested 16 times is Makalioka with a 20% incidence of the disease We consider those lines that consistent have less than 30% incidence of the disease as demonstrating resistance This compares with the susceptible variety Oryzica 3 that consistently has more than 80 90% incidence of disease The most resistant commercial Colombian variety was O Caribe 8 Colombia 1 which is a principal source of resistance for RHBV proved also to be moderately resistant to crinkling disease Several of the lines in VIOFLAR/1999 were resistant to crinkling disease Since crinkling disease was not a criterion when VIOFLAR/1999 was developed it was encouraging to find some moderately resistant lines within the collection While crosses with *O glaberrima* are being made with the goal of developing new sources and varieties with a high level of resistance to crinkle disease rice lines with resistance should also be used in breeding programs In Table 2 the best rice lines are listed and these are potential sources for breeding rice to be more resistant to crinkle disease

Table 1 The Evaluation of *O sativa* and *O glaberrima* Lines Tested for their Reaction to Crinkling Disease

	% of Incidence										
	<10	**11 20**	**21 30**	**31-40**	**41 50**	**51-60**	**61 70**	**71 80**	**81 90**	**91 100**	
% lines	3 7	0 75	2 25	4 5	13 3	16 6	25 3	19 6	14	0	100/
No of materials	10	2	6	12	35	44	67	52	37	0	265

Table 2 The Individual Reactions to Crinkling Disease of the most Resistant Lines Tested

Material	Code	/ of Incidence	No of Replications
O glaberrima	CG10	0 00	3
O glaberrima	CG148	0 00	3
O glaberrima	CG209	0 00	3
O glaberrima	G143	0 00	3
O glaberrima	Glaberrima	0 00	3
O glaberrima	IG10	0 00	3
O glaberrima	TOG4	0 00	3
O glaberrima	TOG6	0 00	3
O glaberrima	TOG7	0 00	3
O glaberrima	TOG5486	0 00	3
Makalioka	MK	19 58	16
IR5	IR5	20 00	3
PSBRC 70		23 33	3
FL00593 6P 9-4P M	166	24 44	3
Caribe8		26 67	3
Colombia1		27 28	15
FL00596-54P 3 2P M	200	27 50	3
FL00593 19P 5 3P M	170	27 78	3
Coprosem1		31 93	27
FL00629 10P 5 3P M	203	32 22	3

Comparing the Effect of Direct Seeding versus Transplanting on the Incidence of Crinkling Disease Two sets of conditions were used to test the effect of transplanting rice on the incidence of crinkling disease One treatment consisted of using contaminated seed and then planting the rice in sterile soil The other comparison started with clean seed and planted the rice into contaminated soils In all cases transplanting increased the incidence of crinkling disease (Table 3) The disease incidence was higher when the source of inoculum was the contaminated soil as compared to contaminated seeds Transplanting rice when the soils were contaminated led to 90% or more infection in all four of the rice lines The most susceptible variety in this experiment was Oryzica 3 as demonstrated by the higher incidence of crinkling disease in the direct seeding treatments The act of transplanting produces small wounds on the roots facilitating the entry of the zoospores and this was the most probable reason for the increase in disease incidence If this was the main effect of transplanting then other factors that cause root lesions such as nematode or aphid damage may also lead to higher incidence of the disease even though the rice was seeded directly Before RSNV was identified as the causal agent of Entorchamiento both nematodes and red root aphids were thought to be associated with crinkling disease While these are not the cause of the disease it is worth investigating if these agents aggravate the disease

Table 3 Testing Methods of Inoculation of Crinkling Disease in Four Rice lines

Seed Treatment	Treatment Type of Seeding		% Incidence/Plant Height in cm			
			FL00478-29 P 18 1P M	FL00518-1 P-4 3P M	Fedearroz 2000	Oryzica 3
Contaminated Seeds	Sterile soil direct seeding	Incidence	0 1	0 1	0 1	45
		Plant height	43 05	39 9	39 9	39 7
Contaminated Seeds	Sterile soil Transplanted	Incidence	5 05	10 05	40	40
		Plant height	43 05	39 9	40 35	39 7
Clean Seeds	Soil contaminated Direct seeding	Incidence	39 15	25 05	34 09	85
		Plant height	44 45	45 7	46 1	36 15
Clean Seeds	Soil contaminated transplanted	Incidence	95	95	95	90
		Plant height	20 6	16 25	26	24 45

1 Incidence was determined at 32 days after planting

Seed Treatments to Control Crinkling Disease A set of experiments was done to determine if a rapid treatment with dry heat could be used to reduce the incidence of crinkling disease Although the treatment of 85° C killed the seed none of the treatments in which the seed was viable had any effect on the incidence of the disease (Table 4) Further testing will be done to determine the effect of other treatments but the resting spores do not appear to be affected by heat treatments and they may be more stable than rice seed Wet heat treatments also will be tested This would have an effect of partially washing the seed and could reduce incidence even if it does not inactive the resting spores Fungicide treatments will also be tested with the idea of inhibiting the zoospores that are released from the resting spores

Table 4 Effect of Temperature Treatments of Seed on the Incidence of Crinkling Disease

Temperature	% of Plants with Crinkling Disease	Dead Plants
26	25 4	0
55	37 9	2
65	28 3	0
75	28 2	2
85		No germination

The Effect of Soils Contaminated with Crinkling Disease on the Roots of Rice In this experiment ten rice varieties and *O glaberrima* were planted in soil contaminated with *P graminis* containing RSNV and in sterile soil Root weight was used as the primary criteria to determine the effect of the treatments There was a statistically significant difference in the root weight of all varieties except Coprosem 2 grown in contaminated soil as compared to those grown in sterile soil (Table 5) When analyzed globally the effect of the soil is independent of the variety and does not correlate with the incidence of crinkling disease Despite relatively low percentage of leaf symptoms in Bluebonnet 50 and Colombia XXI there were a two to three fold decrease in root weight when the plants were grown in the contaminated soil as compared to the sterile soil This was also true for *O glaberrima* despite the fact that none of the plants had leaf symptoms

Table 5 The Effect of Crinkling Disease on the Root growth of Rice

Variety	Dry Weight of Roots (g)/Plant		
	Sterile Soil[1]	Contaminated Soil	% Plants with Leaf Symptoms
Bluebonnet 50	0 037	0 013	17
Yacu 9	0 045	0 023	55
Selectra 320	0 049	0 025	50
O Caribe 8	0 051	0 024	82
Oryzica 3	0 058	0 021	77
Colombia XXI	0 056	0 030	21
Fedearroz 2000	0 060	0 027	63
Coprosem 1	0 062	0 026	42
Coprosem 2	0 032	0 025	70
O Llanos 5	0 074	0 024	78
O glaberrima	0 055	0 030	0
All varieties	0 057	0 025	50

[1] The contaminated soil was a mixture from fields infested with crinkle disease It was sterilized by steam treatment for 2 hours

Few nematodes were found associated with the roots but they had been thoroughly washed The roots were not tested for the presence of *P graminis* although it is known that *O glaberrima* can be a host The aerial portion of the plants that were not infected with RSNV appeared to be similar in size in both treatments although this trait was not measured Additional experiments are needed to understand the large difference in root weight and the biological factors that are influencing the root development How much of the root weight loss was due to infection with *P graminis* versus other biological factors needs to be investigated

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- **Rice Stripe Necrosis Virus Identification of Resistance Sources to the RSNV (Crinkling or Entorchamiento) under Greenhouse Inoculations Evaluation of Wild Species and Progenies Development of Evaluation Methods**

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Abstract

The RSNV disease has been disseminating in the rice growing areas of Colombia at a relatively rapid rate All Colombian commercial rice cultivars are susceptible to the disease High level of resistance to RSNV has been identified in the wild species *O glaberima* We are reporting here the results in transferring the resistance genes from the wild species into susceptible commercial rice cultivars Resistant lines to RSNV have identified and will be tested under natural infection in farmers fields

Introduction

The fungus *Polymyxa graminis* transmits rice stripe necrosis virus (RSNV) or crinkling disease also known as entorchamiento The disease has been disseminating in the rice growing areas of Colombia at a relatively rapid rate in the last few years as a result of the movement of contaminated equipment to non contaminated rice fields Contaminated seed harvested in infected fields is another source of spread Although the virus is not seed transmitted the resting structures of the fungal vector of the virus can be carried on the husk of rice seed in infected debris or soil particles The disease has been reported and confirmed in Panama probably as a result of the introduction of infected seed imported from contaminated areas in Colombia Most of the Colombian rice cultivars exhibit different levels of susceptibility to the virus under field and

greenhouse conditions as reported in previous annual reports We reported last year the identification of high levels of resistance to entorchamiento in the species *Oryza glaberrima* We are reporting here the results in transferring the resistance genes from the wild species into susceptible commercial genotypes of the *O sativa*

Materials and Methods

Several breeding populations were developed from the crosses between the species *O glaberrima* and the rice cultivars Bg90 2 (irrigated) or Caiapo (upland) A total of 1200 and 200 lines from each cross respectively were inoculated and evaluated for their reaction to entorchamiento under greenhouse conditions Inoculum was infested soil collected from farmers fields during epidemic development of the disease in 1999 in the Cauca Valley and Tolima Ten plants per treatment from each line was tested using four different sources of infested soil The highly susceptible cultivar Oryzica 3 was used as a control in all the experiments Incidence of all symptoms typical of entorchamiento including crinkling yellowing or chlorosis stunting and dead plants were evaluated on a weekly basis beginning 20 days after planting

Results

The species *O glaberrima* exhibited high levels of resistance to entorchamiento and less than 1% of the plant had symptoms of the disease (Tables 6 and 7) The susceptible control Oryzica 3 exhibited a high incidence of all symptoms of entorchamiento demonstrating the high inoculum pressure of the soil used for the inoculation (Tables 6 and 7) The susceptible rice cultivars Bg90 2 and Caiapo used in the backcross with *O glaberrima* exhibited lower levels of susceptibility than the susceptible control Oryzica 3 (Tables 6 and 7) The resistance to entorchamiento present in the wild relative has been successfully transferred to the commercial cultivars used in the crosses (Tables 8 and 9) Highly resistant lines were identified and will be inoculated in replicated trials as well as planted in the field under natural infection The number of resistant lines was higher in the BC_2F_5 compared to the more advanced backcrosses BC_3F_4 and BC_3F_5 (Table 8) It should be noted however that this result was expected as the lines selected in each backcross were independent of their reaction to entorchamiento It was possible to identify some lines highly resistant as the resistant parent *O glaberima* Some lines were also more susceptible than the susceptible parents Bg90 2 and Caiapo (Table 9)

Discussion

High levels of resistance to entorchamiento have not been detected in continuous evaluations of the cultivated species *O sativa* On the other hand high levels of resistance have been detected in the wild species *O glaberrima* in several independent trials Transferring the resistance to entorchamiento observed in the wild species to the cultivated species seems to be an easy task according to greenhouse inoculations of two breeding populations evaluated for entorchamiento

resistance The resistance observed need to be confirmed in additional trials both under greenhouse conditions as well as in farmers field under natural conditions of infection

Future activities

The evaluation and selection for entorchamiento resistance using interspecific progenies will continue by additional greenhouse screening The results will be corroborated under natural field infection The genetic control of entorchamiento resistance in the wild species *O glaberrima* will be done using the segregation of the progeny of these crosses

Table 6 Incidence of RSNV (Entorchamiento) of Parents Used in a Backross between *Oryza glaberrima* and the Irrigated Rice Cultivar Bg 90 2

Line	Inoculated Plants	Crinkling /	Chlorosis /	Stunting /	Dead Plants /
O glaberrima	35	0 3	0 7	0 7	0 3
Bg 90 2	80	16	15 2	17 6	12
Oryzica 3 (Susceptible Check)	80	30 4	47 2	44 8	12

Table 7 Incidence of RSNV (Entorchamiento) of Parents Used in a Backross between *Oryza glaberrima* and the Upland Rice Cultivar Caiapo

Line	Inoculated Plants	Crinkling /	Chlorosis /	Stunting /	Dead Plants /
O glaberrima	31	0	0	0	0
Caiapo	26	16	23	31	16
Oryzica 3 (Susceptible Check)	28	57	57	82	18

Table 8 Identification of BC_2F_5 BC_3F_4 and BC_3F_5 Rice Lines of the Cross *Oryza glaberrima* (Acc IRRI 103544) X BG 90-2 Resistant to RSNV (entorchamiento) in Greenhouse Inoculations

	Line Identification		Line Identification
	BC_2F_5	31	CT15150 M 79 21 2
1	CT15150 M 9-4 1	32	CT15150 M 85 5 1
2	CT15150-M 9-4 2	33	CT15150 M 90 5 1
3	CT15150-M 9-4-4	34	CT15150 M 92 3 5
4	CT15150-M 17 1 1	35	CT15150 M 106 5 1
5	CT15150-M 21 8 2	36	CT15150 M 106 5 2
6	CT15150 M 27-4 2	37	CT15150 M 124 1 2
7	CT15150 M 30 2 3	38	CT15150 M 124 3 2
8	CT15150 M 48 3 5	39	CT15150 M 129 1 1
9	CT15150 M 50 2 1	40	CT15150 M 129 1 2
10	CT15150 M 50 2 2	41	CT15150 M 129 1 3
11	CT15150 M 50 2 5	42	CT15150 M 149 1 1
12	CT15150 M 50 3 1	43	CT15150 M 181-4 1
13	CT15150 M 50 3 5	44	CT15150 M 190 2 1
14	CT15150 M 50 3 6	45	CT15150 M 234 7 1
15	CT15150 M 50-4 1	46	CT15150 M 242 3 1
16	CT15150 M 50-4-4	47	CT15150 M 242 3 2
17	CT15150 M 50 5 1	48	CT15150 M 242-4 1
18	CT15150 M 79 7 1	49	CT15150 M 249 5 1
19	CT15150 M 79 7 2	50	CT15150 M 249 5 2
20	CT15150 M 79 9 3		
21	CT15150 M 79 9-4		
22	CT15150 M 79 10-4		**BC_3F_4**
23	CT15150 M 79 11 2	1	CT16053A 6 1 1
24	CT15150 M 79 11 3	2	CT16070A 2 1 1
25	CT15150 M 79 11-4	3	CT16101B 1 1 1
26	CT15150 M 79 15 1	4	CT16148 1 9 1
27	CT15150 M 79 15 2		
28	CT15150-M 79 16 1		
29	CT15150-M 79 18 2		**BC_3F_5**
30	CT15150 M 79 19 2	1	CT16049A 7 2 1 1

Table 9 Rice Lines of the cross *Oryza glaberrima/*Caiapo Resistant to RSNV (Entorchamiento) in Greenhouse Inoculations

	Pedigree	Crinkling (/)
1	CT 16308 CA 5	0
2	CT 16308 CA 6	0
3	CT 16311 (2) CA 3	0
4	CT 16313 –CA 5	3
5	CT 16310 (2) CA 6	3
6	CT 16313 CA 16	3
7	CT 16307 (1) CA 7	4
8	CT 16307 CA 1	4
9	CT 16307 (1) CA 2	4
10	CT 16310 (2) CA 1	4
11	CT 16316 CA 7	6
12	CT 16318 CA 1	6
13	CT 16308 CA 4	6
14	CT 16308 CA 7	7
15	CT 16310 (2) CA 5	7
16	CT 16312 (2) CA 6	7
17	CT 16307 CA 4	8
18	CT 16315 (1) CA 14	8
19	CT 16315 (1) CA 17	8
20	CT 16316 CA 2	8
21	CT 16322 CA 7	8
22	CT 16322 CA 5	9
23	CT 16323 CA 6	9
24	CT 16322 CA 6	9
25	CT 16313 CA 3	10
26	CT 16312 (2) CA 3	10
Highest susceptible line	CT 16307 CA 12	53
Parent 1	*O glaberrima*	0
Parent 2	Caiapo	16
Susceptible check	Oryzica 3	57

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

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 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

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

- **Confronting Food Insecurity in the Hillsides**

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Abstract

The crosses of a diallele for the genetic study of cold tolerance are in way The progenitors used as cold tolerance come from different continents

During the first semester in the Colombian hillsides a tremendous drought due to the *Nina* phenomenon dried all the trials In spite of that in the experimental station of Popayan (1 700 m asl 2 N) Cauca Colombia it was possible to observe the good comportment of YUNLU 29 and Basmati C621 during the vegetative stage in the evaluation trial of introduced varieties and potential progenitors Also it was possible to harvest few plants in the populations with narrow genetic base PCT 14 (lowland) and PCT 17 (upland) in the participatory improvement of populations Finally in this station 75% of the lines were recovered what will allow to select promissory lines with tolerance to the cold due to the altitude early (150 days sowing harvest)

and with drought resistance In the participatory evaluation trial carried out in the country side for some communities it was possible harvest few rice lines in particular the advanced lines PRA553 44 64 1 1 and PRA553 45 8 8 1 from IRAT 265 57 2 / Jumli Marshi

The first workshops on participative development of rice for hillsides were carried out 19th and 20th September 2001 for the Caucan rural communities took place in the CETEC Green School Santander de Quilichao Cauca Colombia and the 7th November 2001 workshop for *Vallunas* rural communities took place in the Yumbo UMATA Valle Colombia

Introduction

The mid altitude hillsides such as the highlands of Madagascar constitute a marginal area for the upland rice crop because of the cold nighttime temperatures Often the populations of these area live in communities which tend to be neglected Often the population is predominately an ethnic minorities as in Colombian Andes (Guambianos Totoroes Coconucos etc) or in the Himalayan part of Yunnan in China (Xi Yao Lagu Dai etc) These areas although geographically diverse have similar climate and social challenges Therefore collaborations have been established with Madagascar (Dzido 2001) China (Vales 2000 and 2001c) and Colombia (see below) We expect to start working in Central America next year and this work could easily be adapted to the Amazon region

The following activities have the financial support of the French Foreign Office (*Ministere des Affaires Etrangeres*) the Foundation Aventis Institute of France CIRAD and CIAT

The objective is the genetic improvement to obtain varieties well adapted to the agro climatic conditions and preferences of the producers and consumers using participatory methods Since varieties is not the only limiting factor often work on the production system or post harvest processing is requested by the farmers groups and becomes a part of these projects

Materials and Methods

Evaluation of Introduced Varieties and Potential Progenitors

Forty five YUNLU lines from the Yunnan Academy of Agricultural Sciences (YAAS) 18 European varieties provided by the French Rice Center (French acronym CFR)/CIRAD and 6 checks were used The checks were T1 F8 218 119 (IRAT 146 / Daniela) T2 C8 F322 8 8 5 3 (Latsidahy / IRAT 351) T3 Shinei T4 Latsibavy T5 CIRAD 407 T6 CENICAFE

This activity was carried out in the Popayan sub station Cauca Colombia 1 700 m asl 2 N during the first semester 2001 (PSS) The trial design was 4 complete randomized blocks of 69 lines and checks Four 3 m rows (0 25 m interval) were sown by lines One seed was sown at a distance every 0 125 m interval along the row That allowed a low density of 10 kg/ha needed to produce enough seeds to meet the community requirements

Participatory Improvement of Populations

Five populations with cold tolerance were tested These included two populations developed for Madagascar and are called Lowland MGD and Upland MGD The PCT 13 population with broad genetic base the PCT 14 (lowland) and PCT 17 (upland) populations with narrow genetic bases were chosen for the partipatory improvement of populations The trial was carried out in the PSS during the first semester 2001 For both the Lowland MGD and the Upland MGD populations 5 000 plants were sown For the both populations PCT 14 and PCT 17 1 000 plants were sown For the population PCT 13 10 000 plants were sowed in PSS and 1 000 plants in each of the 10 Caucan villages of rural associations network (ARDECAN) of the Corporation for multidisciplinary studies and technical (Spanish acronym CETEC) One of the purposes of this dual approach was to compare the participatory selection (producers in experimental station and in their villages) with convention selection by the investigator on an experimental station

Creation of varieties

The population consisted of 1 209 lines F4 and 946 lines F5 from cold tolerant population Eleven varieties were used as checks including T1 Latsibavy T2 CIRAD 407 T3 Rojofotsy Vin T4 CIRAD 391 T5 Estrela T6 Shinei T7 CENICAFE T8 CIRAD 396 T9 Raksali T10 F8 218 119 (IRAT 146/Daniela) and T11 C8 F322 8 8 5 3 (Latsidahy/IRAT 351)

The pedigree selection was carried out at PSS during the first semester 2001 The trial design was in 77 Federer s blocks of 28 lines and 11 checks Two rows of 3 m were sown by line The intervals were 0 25 m between rows and 0 125 m between seeds (10 kg/ha)

Results

Evaluation of Introduced Varieties and Potential Progenitors

Due to an unusally drought the trial was dried before the heading stage (Figure 1) It was just possible to observe the good comportment of YUNLU 29 and Basmati C621 during the vegetative stage This is normally a high rainfall area and this years experience shows the challenges facing upland rice It needs not only to be cold tolerance but it must be able to withstand both substantial rainfall and dry conditions Drought tolerance will become a priority

and better water control on a farm level is also important to assure food security

Participatory Improvement of Populations

Due to the severe drough all the populations preformed poor with premature drying of the plants It was only possible to harvest few plants in the populations with narrow genetic base PCT 14 (lowland) and PCT 17 (upland) These seeds that were from plants that withstood unusually dry conditions and the seeds will be tested for drought tolerance

Creation of Varieties

In spite of the tremendous drought at PSS 75% of the lines were recovered After the analysis of the data the most promising lines will be selected The characteristics that are being used for the selection include tolerance to the cold precocity (150 days sowing harvest) and drought resistance

- **Genetic Study of Cold Tolerance and Creation of Variability**

Materials and Methods

To efficiently use the source of cold tolerance it is important to know the genetic inheritance of this character The study of diallele crosses progenies was the made to study the genetic inheritability of cold tolerance The progenitors for cold tolerance have different genetic origin (Table 1)

Table 1 *Diallele Progenitors*

Variety	Type	Origin
Jumli Marshi	Lowland japonica	Nepal
Latsibavy	Lowland japonica	Madagascar
Yunlu 29	Upland japonica	China
Kunming Xibaigu	Lowland japonica	China
Maon	Lowland indica	China
Kulon	Lowland japonica	Russia
Sandora	Lowland japonica	Hungria

Oro	Lowland japonica	Chile
Artiglio	Lowland indica	France
Basmati C621	Lowland basmati	India

The progenies of the diallele crosses will be part of the breeding program as other descendants and new recurrent population with narrow genetic base (see Output 1)

Results

The program of crosses is in progress

Participatory Evaluation of Advanced Lines and Varieties

Materials and Methods

Fifty two participatory trials were sowed in 26 locations as following

1 Corporation for Interdisciplinary Studies and Technical Consultantship (Spanish acronym CETEC) 20 trials with 12 associations
2 CIAT Hillsides Project 13 trials with 7 CIALs
3 International Center for Organic Agriculture (Spanish acronym CIAO) 11 trials with 4 villages
4 Municipal Unit for Agricultural Transfer (Spanish acronym UMATA) of Yumbo Valle (Colombian Ministry of Agriculture) 6 trials with 2 associations
5 Caldas University 2 trials with 1 village)

The locations are not accessible due to the insecurity problem so two meetings with the farmers were organized in March in the CETEC Green School of Santander de Quilichao Cauca Colombia The first one was to know what the farmers want and the second one was to demonstrate how put in place the trials In September a two days workshop with the farmers was carry out to talk about the results and our followed collaborative activities

Seventeen F_7 lines of upland rice for hillsides i e with cold tolerance were also provided to various partners (Table 2)

Table 2 Partners for Upland Rice for Hillsides, and/or for Cold Tolerance

Country	Institution
Chile	INIA
China	YAAS/FCRI
Colombia	FEDEARROZ
Costa Rica	UCR/DESARROZ
France	CIRAD CFR
Haiti	MARNDR
Honduras	CIAT
Madagascar	CIRAD FOFIFA
Nicaragua	CIAT

Results

Due to this drought the communities had loss the main part of all their crop production The rice trials were in general completely dried But for some communities it was possible to harvest few rice lines in particular the advanced lines PRA553 44 64 1 1 and PRA553 45 8 8 1 from IRAT 265 57 2 / Jumli Marshi

So during the workshop with the farmers 19th and 20th September seeds multiplied in Palmira station were provided to them for immediate sowing taking advantage of the beginning of the rain of the second semester

General discussion and Perspectives

In spite of a tremendous drought some plants and promissory lines were selected in experimental station or by the farmers in their field So it is possible to follow the breeding process with this material which has a good tolerance to the cold due to the altitude often very early (to 150 days sawing harvest) and a strong drought resistance

To not narrow too much the genetic base of the breeding program for hillsides rice it will be possible to use again the same recurrent populations multiplied in the Palmira experimental station

The same lines and populations were provided to the University of Costa Rica /Rice Development Project (Spanish acronym UCR/DESARROZ) (Vales 2001b) and to the Yunnan

Academy of Agriculture Sciences (YAAS) of P R of China YAAS wants extend its collaboration with a new project on rice hybrids for cold hillsides (Vales 2000 2001) The use of the new methods to breed resistance to rice blast disease is part of this activity (see Output 2)

The Ministry of agriculture natural resources and rural development (French acronym MARNDR) of Haiti wants develop a collaboration to reactivate the rice crop in the hillsides of this country (Vales 2001a)

Reference

Dzido J L 2001 CIRAD Annual Report for 2000

- **Reactivation of Rice Crop on the Coasts**

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Abstract

Rice production has been decreasing on the Timbiqui river strands This project provided commercial seeds to increase the rice growing areas During the first semester much of the rice productions including the new fields on the Timbiqui river strand were destroyed by an inundation in May During the second semester 50 rice fields were planted by the College of Agriculture of Santa Rosa the Primary School of Puerto Luz and women associations of the Matomba and Guasa network with the commercial seeds provided by this project This is expected to be the beginning of an expansion of the rice crop in the river bank system From the 4 traditional varieties Fian Brillalola Chino Grande and Chino Chiquito coming from the Guapi river strands more than 20 different types of rice were observed and selected This activity is rescuing traditional varieties Since the trials for the participatory evaluation of rice

varieties were lost during the first semester they trials were sowed again and will soon be ready for evaluation and harvesting However in the early results some promising yields were reported The main criterions for the producers in order of priority are precocity grain shape and production A motorized huller prototype was evaluated and failed by communities A second prototype has been produced and is being evaluated A survey to understand the preference of the rice producers and consumers is being made by the women association network Mundo Afro
Due to the high rainfall low light and salinity conditions of the area additional breeding efforts have begun that targets these conditions

Introduction

Few years ago due to development projects of oil palm tree coconut tree production and logging the communities of the pacific coast of the Colombian Cauca Department their traditional production of rice declined These development projects have had limited success because of a drastic decrease in the coconut price and disease of the oil palm trees As a consequence there is a growing demand for the important food crops In areas such as the Timbiqui river strands so little rice was grown that 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) 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

The immediate objective is the production of enough rice that the rural communities are self sufficient for this basic food stable Then nest objective is the production of enough rice to supply the regional markets thereby make both the farmers and consumers better off The first step is to reactivation the production but supplying commercial seed to the resource poor farmers The next step to select better varieties either through the rescue the traditional varieties or through the introduction of addition diversity This is being done in a participatory manner Besides the farmer participation in the selection of rice varieties their needs such as low cost small scale equipment for the dry and hulling of rice are being given priority If successful the community has more economic viability and this project will be expanded to similar types of areas

Materials and Methods

- **Rice Crop Reactivation on the Timbiqui River Strands**

Some farmers have in memory the good comportment in trial of the variety Oryzica Caribe 8 So one ton of commercial seeds of O Caribe 8 were provided to reintroduce rice on the Timbiqui river strands The recipient communities have to pay back to the UMATA seed bank with twice the amount of seed provided to them

Participatory Rescue of Traditional Varieties on the Guapi River Strands

In June 2000 samples of the varieties Fian and Brillalola were provided by the seed bank of the Guapi UMATA This material was sown and seeds from individual plant types were selected In February 2001 the varieties Chino Grande and Chino Chiquito were grown in farmer fields on the Guapi river strand and the seed was collected from different plant types The seeds of these plants were grown in order to rescue tradition cultivars that are now part of the mixture of these local varieties

Participatory Evaluation of Rice Varieties

Fifteen varieties were evaluated by 5 communities on the Guapi river strands and 5 communities along the Timbiqui river The check variety was Oryzica Caribe 8 during the first semester and FEDEARROZ 2000 was the check during the second semester The crop management was traditional and organic without the use of any chemicals

The trial design was the collection with an intercalated check variety to allow an interpretation for each locality The different individual trials form a multi location design in complete randomized blocks The criterions for selection were agronomic traits and the consumers preferences of taste and cooking comportment

A lowland rice recurrent population PCT 6 was evaluated in farmer s fields for the selection of the best adapted plants Lowland varieties with salinity tolerance were crossed with the best materials from the PCT 6 population to form an adapted recurrent population for the Pacifica coast region of Colombia

Participatory Rice Huller Evaluation

An Engelberg type rice huller plan from IRRI was provided to the Metalica Metropolitana to make prototypes These prototypes are to be evaluated by communities

Participatory Survey on the Traditional Production of Rice

With the women association network Mundo Afro a survey is being carrying to better understand the traditional rice management practices and the consumer preferences

Results and Discussion

Rice Crop Reactivation on the Timbiqui River Strands

During the first semester commercial seeds of the variety Oryzica Caribe 8 were distributed through community organization in the Timbiqui river strands Most of the fields in the area were flooded in May and all of the introduced materials were destroyed The disaster affected not only the project Three thousands people were disaster victims and national food help was made available During the second semester 50 rice fields were grown by the College of Agriculture of Santa Rosa the Primary School of Puerto Luz and women associations of the *Matomba* y Guasa network This semester the fields are in good shape and normal rice production is expected

Participatory Rescue of Traditional Varieties on the Guapi River Strands

From the 4 traditional varieties Fian Brillalola Chino Grande and Chino Chiquito more than 20 different types of rice were observed This indicates that these varieties were a mixture of traditional lines To rescue these lost traditional cultivars seeds were collected from individual plants of each plant type that was observed in the fields These different lines are now being grown multiplied and evaluated as a source of local materials that are adapted to the conditions of the Pacific coast

Participatory Evaluation of Rice Varieties

During the first semester lots of trials were destroyed by the inundation disaster of May mainly along the Timbiqui river So the trials were sowed again On the Guapi river strands rice fields exist so the bird pressure was not too strong Some of the varieties are not yet harvested

however the best observed production is 4 5 t/ha which was good compared with the yields that are normal using these cropping conditions The main criterions for the producers were in rank precocity grain shape then production It appears that the best adapted materials are the traditional varieties

There is currently little rice production in the Timbiqui river strands and pressure by birds feeding on the crop prevented the tabulation of accurate production data The producers spent their limited resources exclusively for the new production fields and ignored tending to the participatory trials During the next semesters these trials will be done in the same the production fields and more efforts are needed to have the farmer s adopt these participatory trials The best adapted varieties at the early stage are the traditional ones

Due to the lack of light and salinity problem the lowland rice recurrent population PCT 6 performed poorly and is clearly not adapted for this area Nevertheless a few plants of this population were satisfactory and will be recombinated to make the next generation recurrent population and then fixed lines will be selected to produce the advanced lines (see Output 1)

Participatory Rice Huller Evaluation

A prototype huller with a gasoline engine was made by the Metalica Metropolitana and evaluated in several communities The huller did not function well in the rain forest conditions where the seeds stay very wet Much of the grain shattered and the moter become hot too quickly A second prototype has been made and is ready to be evaluated These communities also need better ways of drying the grain and will be part of the development of small inexpensive machinery to aid small resource poor farmers

Participatory Inquiry on the Traditional Production of Rice

To better understand the community and their preference as rice producers and consumers a survey is being carried out by the women association network Mundo Afro The results will be used for the orientation of the collaborative trials and the breeding program for producers and consumers

Perspectives

The rice project for the Pacific coast region of Colombia on the Timbiqui river strands has proven to be a challenge Seed production is expected to increase as a result of the UMATA seed bank system More or less 20 different types of plants are in way of fixation and this will allow the recovery of lost traditional rice varieties Evaluate of hullers including inexpensive two pass hullers from China is in progress

3B CIAT – FLAR Collaboration

CIAT in cooperation with other FLAR members provides important resources to rice research in the region Most importantly these resources remain as international public goods Needless to say some of FLAR s outputs are restricted in nature and are available to institutions in member countries However there are several activities that remain public and receive close support from CIAT s rice project staff They include the maintenance of the Working Collection (or germplasm bank) the interchange of elite rice germplasm through INGER LAC nurseries the provision of specific services in the characterization of lines and varieties and activities related to information dissemination and training of scientists throughout the region

This year the working collection went from 1742 to 1987 entries which are kept under special conditions for long term storage thanks to a recent remodeling of the storage facility at CIAT These entries are commercial varieties as well as interesting lines for irrigated and upland rice which are thoroughly characterized for a number of relevant traits such as resistance to rice blast rice Hoja Blanca virus Sogata planthoppers cold tolerance and several quality characteristics (both milling and consumption traits)

INGER LAC The main source of material for the INGER LAC nurseries is IRRI (INGER global) FLAR distributes the nurseries under the name of VIOAL (observation nurseries for Latin America) This year VIOAL 2000 consisting of 60 entries went to 9 countries in the region and four of them (Ecuador Paraguay Dominican Republic and El Salvador) are non FLAR members The VIOFLAR 2001 was conformed by 54 entries that came from INGER global (28 of them) Peru (5 entries) Cuba (2 entries) CIAT (14 entries) and older VIOALs (5 entries) For the VIOAL 2002 we have 70 entries from Ecuador plus the accessions from IRRI CIAT and CIRAD INGER remains a prime mechanism for germplasm interchange in the region For countries outside FLAR membership is the main source of foreign germplasm CIAT and FLAR s role in maintaining this service in cooperation with IRRI is seen as a strategic activity

Other activities were the characterization of lines relate to RHBV Sogata quality and blast Between the second semester of 1998 and the first semester of 2001 over 20 000 FLAR lines were tested for rice Hoja Blanca virus Over 21 000 lines were tested for quality (white belly grain appearance and length amylose content) Half of those lines are from CIAT s breeding program and the rest are from FLAR FEDEARROZ ICA and entries from several countries In Santa Rosa a total FLAR planted a total of 5 145 lines for evaluation The station is a strategic hot spot for blast In addition to that 12 progenitors for the cold breeding program were characterized at CIAT

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

3C Cooperators Training and Information

- **Cooperators**

Latin America and the Caribbean

FLAR

Luis R Sanint Executive Director
Peter R Jennings Consultant
Edward Pulver Consultant
Carlos Bruzzone Consultant
Luis Eduardo Berrio Research Associate
Maribel Cruz Research Assistant

Argentina
Marta Nicosia University of Tucuman
Alberto Villegas University of Tucuman
Juan Eduardo Marassi University of La Plata
Alfonso Vidal University of La Plata
Alberto Livore INTA

Bolivia
Roger Taboada CIAT Santa Cruz de la Sierra
Jorge Rene Guzman CIAT Santa Cruz de la Sierra

Brazil
Elcio Perpetuo Guimaraes EMBRAPA Rice and Beans Center Goiania

Chile
Santiago Hernaiz INIA Quilamapu Chillan
Roberto Alvarado INIA Quilamapu Chillan

Colombia
Hernando Delgado CORPOICA La Libertad Meta
Edgar Corredor FEDEARROZ Saldana Tolima
Luis Reyes FEDEARROZ Saldana Tolima
Javier Osorio University of Tolima
Miguel Diago FEDEARROZ Santafe de Bogota

Patricia Guzman FEDEARROZ Ibague
Roberto Simmonds Cultivos y Semillas El Aceituno Ltda Ibague
Nestor Ramos Semillano Ltda Villavicencio Meta

Cuba
Luis Aleman Instituto de Investigaciones del Arroz (IIA)
Rene Perez Polanco Instituto de Investigaciones del Arroz (IIA)
Ruben Alfonso Instituto de Investigaciones del Arroz (IIA)
Alfredo Gutierrez Viceministro de Agricultura Ministerio de Agricultura
Nelson Gonzalez Director UAIA Ministerio de Agricultura
Jorge Hernandez Instituto Investigaciones del Arroz (IIA)

Dominican Republic
Cesar Moquete Instituto Dominicano Investigaciones Agricolas y Forestales (IDIAF)

Uruguay
Fernando Perez de Vida INIA Treinta y Tres (currently in the U S)
Pedro Blanco INIA

USA
Susan R McCouch Cornell University USA
James Oard Louisiana State University USA
Anna McClung Texas A&M University USA
Robert S Zeigler Kansas State University USA

Venezuela
Gelis Torrealba Instituto Nacional de Investigacion Agricola (INIA former FONAIAP)
Carlos Gamboa DANAC Foundation
Ramiro de la Cruz DANAC Foundation
Eduardo Graterol DANAC Foundation (currently in the U S)
Edgar Torres FUNDARROZ

Africa
Monty Jones WARDA – Cote d Ivoire –
Gabriel Aluko PhD student WARDA

Asia
IRRI – The Philippines –
Gurdev S Khush
Peter Kerridge CIAT Asian Office LAOS

China
Tao Dayun Food Crop Research Institute of the Yunnan Academy of Agricultural Sciences (FCRI / YAAS – Yunnan Province)
Lee Kai Mian Chinese Academy of Tropical Agricultural Sciences (CATAS Hainan Province)

Europe
Guy Clement CIRAD – France
Gilles Trouche CIRAD– France
Didier Tharreau CIRAD France
Emmanuel Guiderdoni CIRAD France
Alain Ghesquiere IRD

Training

- **Thesis**

1 Johanna Patricia Villamizar Ruiz Tesis de Pregrado 4 Identificacion de cambios geneticos en el hongo *Pyricularia grisea* Sacc Asociados a la perdida de resistencia en lineas/variedades de arroz *Oryza sativa* Presidente de Tesis Dr Fernando Correa Universidad Nacional Seccional Palmira Mayo 2001 Mayo 2002
2 Sandra Patricia Valdez Gutierrez Tesis de Pregrado Caracterizacion de germoplasma de arroz y poblaciones derivadas de cruces interespecificos a la resistencia al virus de la necrosis rayada (RSNV) Presidente de Tesis Dr Fernando Correa Universidad Nacional Seccional Palmira Abril 2001 Abril 2002
3 Johanna Echeverry Rico Tesis de Pregrado Caracterizacion de germoplasma de arroz y poblaciones derivadas de cruces interespecificos a la resistencia al virus de la necrosis rayada (RSNV) Presidente de Tesis Dr Fernando Correa Universidad Nacional Seccional Palmira Abril 2001 Abril 2002
4 Gustavo Adolfo Prado Patino Tesis de Maestria Estudio de la herencia de la resistencia de las lineas isogenicas (C101 LAC y C101 A51) y uso de marcadores moleculares asociados a la resistencia Presidente de Tesis Dr Fernando Correa Universidad Nacional Seccional Palmira Enero 2001 Diciembre 2002
5 Fabio Escobar Rioja Tesis de Maestria Caracterizacion de la estructura genetica del patogeno del arroz *Rhizoctonia solani* Presidente de Tesis Dr Fernando Correa Universidad Nacional Seccional Palmira Enero 1999 Julio 2002
6 Yolima Ospina Tesis de Maestria Evaluation of genetic progress for acid soil tolerance and different agronomic characteristics Presidente de Tesis Dr Marc Chatel Universidad Nacional Seccional Palmira Abril 1998 Abril 2002
7 Johanna Paola Dossman Tesis de Maestria Evaluacion del progreso genetico obtenido por un ciclo de seleccion recurrente en la poblacion PCT 6 para la resistencia durable a *Pyricularia grisea* Sacc Y otros caracteres agronomicos Presidente de Tesis Dr Michel Vales Universidad Nacional Seccional Palmira Enero 2001 Diciembre 2003
8 Sandra Milena Salazar Erazo Tesis de Maestria Estudio genetico a traves de cruzamientos dialelicos para resistencia al frio de variedades de arroz para condiciones de ladera Presidente de Tesis Dr Michel Vales Enero 2001 Diciembre 2003
9 Jaime Arias Pasantia de novena (financiamiento opcion colombia) Tesis de Pregrado Reactivacion del cultivo de arroz en los municipios de Guapi y Timbiqui en la costa Caucana (Proyecto PRONATTA Convocatoria 2000) Presidente de Tesis Dr Michel Vales Universidad de Caldas (Director del Grupo de Investigacion Dr Bernardo Rivera) Agosto

2001 Noviembre 2001

10 Peng Xu Capacitacion (financiamiento CIRAD Desi) como parte de la asesoria del proyecto colaborativo CIAT CIRAD a la YAAS dentro del Proyecto Aventis Institut de france Tesis de Maestria Metodos de seleccion para la resistencia durable a la Pyricularia del Arroz Aplicacion en seleccion recurrente para el arroz de laderas Presidente de Tesis Dr Michel Vales Yunnan Academy of Agriculture Sciences (YAAS) R P China R P China Mayo 15 Junio 16 2001

11 Paola Ruiz Tesis de Pregrado Caracterizacion fenotipica y genotipica de los diferentes tipos de arroz rojo (*Oriza sativa*) del Departamento del Tolima Ecotipos de granos largos y delgados con o sin arista Presidente de Tesis Dra Zaida Lentini Universidad Javeriana de Bogota Julio 2001 Julio 2002

12 Juan Jose Vasquez Tesis de Pregrado Caracterizacion fenotipica y molecular de arroz rojo procedente del Tolima Ecotipos de granos cortos y anchos con o sin arista Presidente de Tesis Dra Zaida Lentini Universidad de los Andes Bogota Agosto 2001 Agosto 2002

13 Monica Triana Tesis de Maestria Molecular markers associated with resistance to *Sagotodes oryzicola* M Presidente de Tesis Dr Cesar Martinez Universidad Nacional Seccional Palmira Febrero 2001 Julio 2003

14 Silvio James Carabali Tesis de Maestria Genetic gains in rice grain quality obtained through several cycles of recurrent selection Presidente de Tesis Dr Cesar Martinez Universidad Nacional Seccional Palmira Febrero 2000 Diciembre 2002

15 Jose Alejandro Vargas (FEDEARROZ) Tesis de Maestria Evaluation of two breeding schemes in breeding for yield potential Presidente de Tesis Dr Cesar Martinez Universidad Nacional Seccional Palmira Febrero 2001 Febrero 2003

16 Andrea Maria Garavito E Tesis de Maestria Evaluacion del mecanismo de resistencia viral de una planta transgenica de arroz Presidente de Tesis Dr Lee Calvert Universidad de los Andes de Bogota Julio 2001 Julio 2002

- **Training Courses**

1 First International Congress of Management Rice Cultivar in Dominican Republic 6 10 August 2001 Rafael Meneses and Gustavo Prado

2 Management Rice Cultivar in Villavicencio Meta 17 19 October 2001 Participants CIAT CORPOICA and FEDEARROZ

3 National Breeder s Course Held in Sancti Spiritus Cuba June 18 2001

- **Formation and Training**

1 The Agriculture College of Santa Rosa the Primary School of Puerto Luz and the Primary School of La Magdalena Timbiqui Cauca are participants of this project Commercial seeds are provided to these institutions to reintroduce the rice crop on the Timbiqui strands They use the rice production for the children formation and for obtaining resources

2 The first workshop on participative development of rice for hillsides contributed through information sharing to the farmer formation for rice cropping management The 19th and 20th September 2001 workshop for the Caucan rural communities took place in the CETEC

Green School Santander de Quilichao Cauca Colombia

3 The 7th November 2001 workshop for *Vallunas* rural communities took place in the Yumbo UMATA Valle Colombia

- **Workshops**

1 First International Upland Rice Workshop Villavicencio Meta – Colombia August 7 11 2000 Organized by CIRAD/CIAT Colombia and EMBRAPA Brazil
Participants
Argentina (Universidad Nacional de Tucuman)
Bolivia (CIAT Santa Cruz)
Brazil (EMBRAPA Arroz e Feijao)
Colombia (CORPOICA CENICAFE and University of Tolima)
Cuba (IIA)
Venezuela (INIA)

2 Second International Upland Rice Workshop To be held in Santa Cruz de la Sierra Bolivia in February 2002 Organization during 2001 by CIAT Santa Cruz Bolivia the Japanese Cooperation JICA Bolivia CIRAD/CIAR Colombia and EMBRAPA Brazil

3 I Taller de Seleccion Recurrente en Arroz de Riego en Venezuela To be held in October 29 31 2001 Organization Fundacion DANAC Venezuela CIAT/CIRAD and EMBRAPA

Information

- **Refereed Publications**

1 Quantitative trait loci for yield and yield components in an *Oryza sativa* by *Oryza rufipogon* BC F population evaluated in an upland environment P Moncada C P Martinez J Borrero M Chatel H Gauch Jr E Guimaraes J Tohme S R McCouch Theor Appl Genet (2001) 102 41–52

2 Prado G A Correa Victoria F J Aricapa G Tulande E y Escobar F 2000 Hipotesis de la exclusion de linajes una alternativa para el desarrollo de cultivares de arroz con resistencia durable a *Pyricularia grisea* en Colombia Fitopatologia Colombiana 23 (2) 54 58

3 Prado G A Correa Vistoria F J and Aricapa M G 2000 Estudios sobre la fertilidad y compatibilidad sexual del hongo *Pyricularia grisea* y sus implicaciones en el desarrollo de resistencia durable en variedades de arroz para Colombia Fitopatologia Colombiana 24(2) 55 60

4 Seebold K W Datnoff L E Correa Victoria F J Kucharek T A and Snyder G H 2000 Effect of silicon rate and host resistance on blast scald and yield of upland rice Plant Disease 84 871 876

5 Seebold K W Kucharek T A Datnoff L E Correa Victoria F J and Marchetti M A 2000 The influence of silicon on components of resistance to blast in susceptible partially resistant and resistant cultivars of rice Phytopathology 91 63 69

- **Book Chapters**

Correa Victoria F J Datnoff L E Okada K Friesen D K Sanz J I and Snyder G H 2001 Effects of silicon fertilization on disease development and yields of rice in Colombia p 313 322 In Silicon in Agriculture Ed L E Datnoff G H Snyder and G H Korndorfer Elsevier Science

- **Other Publications**

1 El Arroz de secano nueva opcion de cultivo alimenticio para la region cafetera de Colombia Evaluacion agronomica del sistema de produccion de Arroz intercalado con siembras nuevas de cafe Argemiro Miguel Moreno Berrocal and Chatel M
2 Eastern European Rice Genetic Resources for Rice breeding improvement in France Clement Guy Châtel Marc Chantereau Jacques Feyt Henri Louvel D Seguy Jean Louis and Tharreau Didier
3 Composite Population Breeding using Recurrent Selection in Chile Santiago Ignacio Hernaiz L Jose Roberto Alvarado A Marc Chatel and Yolima Ospina
4 Proceedings of the International Rice Symposium on Genetic Resources and Breeding for Europe and Temperate Area Krasnodar Russian Federation 3 8 September 2001 (Submitted for Publication)
5 Zeigler R S and Correa Victoria F J 2000 Applying *Magnaporthe grisea* population analyses for durable rice blast resistance In Pathogen population genetics and breeding for disease resistance APSnet feature July 1 July 31 2000
6 Peever T L Zeigler R S Dorrance A E Correa Victoria F J and Martin S 2000 Pathogen population genetics and breeding for disease resistance APSnet feature July 1 July 31 2000

- **IP 4 Web Page Development**

With support of the Communications Unit IP4 has worked on the development of our web page This is being done using the new page format designed to give CIAT a uniform web page outlook The rice project web page includes information on our products services and information important to those involve in both research or in rice production It also contains the last three annual reports in English and Spanish posters and guidebooks all in PDF format that these can be downloaded by interested parties

Some of the products offered include catalogs on improved germplasm the FLAR/CIAT Germplasm Bank information on the latest publications and an interactive map of Latin America with the released varieties by country that lists their main varietal characteristics

On access the page shows the latest News regarding events advances and relevant developments in rice research at CIAT and the world

Still under construction is a database of donors and contacts of collaborators which will be finished in 2001

Future Challenges

- Gallery of rice pictures by themes
- An online system to request specific services provided by IP4 such as analyses of samples for Rice Hoja Blanca Virus testing for resistance to *Tagosodes* RHBV rice blast and seeds for quality
- List of Rice Publications by project personnel during the past decade (in a joint effort with CIAT s library)
- A complete database of the rice economy for LAC countries (jointly of the Impact Unit of CIAT)

CIO/CIAT Meeting

1 Every two years takes place a meeting between CIAT and the 3 French Research Institutions CIRAD IRD (former ORSTOM) and INRA This is the forum for reviewing on going and new projects
2 The on going project on Population Breeding was confirmed for two more years
3 A new collaborative project on Participatory Rice and Sorghum Breeding between CIRAD and CIAT was approved CIRAD will outpost a senior breeder and CIAT will finance the operational funds Follow up of the project is to be done previous the end of 2001 for effective starting early 2002

ANNEX 1 PRINCIPAL AND SUPPORT STAFF

Principal Staff

Lee Calvert *Virologist*
Marc Chatel *Plant Breeder CIRAD CA*
Fernando Correa, *Plant Pathologist and Project Leader*
Zaida Lentini *Plant Breeder*
Cesar P Martinez *Plant Breeder*
Rafael Meneses *Visiting Scientist I I A Cuba*
Luis R Sanint *Agricultural Economist Economist and FLAR Executive Director*
Michel Vales *Plant Pathologist CIRAD CA*

Support Staff

- **Associates and Assistants**

Jaime Borrero *Genetics*
Diana Delgado *Genetics (VVC)*
Myriam Cristina Duque *Biometry*
Joanna Paola Dossman, *Pathology CIRAD CA*
Fabio Escobar *Biotechnology*
Ivan Lozano *Virology*
Maria Nelly Medina, *Leader*
Adriana Mora *Genetics/Anther Culture*
Yolima Ospina, *Genetics*
Gustavo Prado *Pathology*
James Silva, *Biometry*
Monica Triana, *Entomology*
Edgar Tulande *Pathology (VVC)**

- **Visiting Scientist**

Edgar Torres DANAC Venezuela
Luis Antonio Reyes Fedearroz Colombia

- **Secretaries**

Elizabeth Hurtado *Leader*
Liliana Escobar *Leader*
Maria Claudia Garzon *Leader*

- **Technicians and Field Support**

Felix Acosta *Genetics*
Tomas Agrono *Genetics/Anther Culture*
Maria Girlena Aricapa *Pathology*
Jesus E Avila *Physiology (VVC)*

Jairo Barona *Leader*
Silvio James Carabali *Genetics*
Efren A Córdoba *Entomology*
Gerardo A Delgado *Genetics*
Jaime Gallego *Genetics*
Jairo Garcia, *Biotechnology*
Victor Hugo Lozano *Genetics (VVC)*
Maria C Martinez *Virology*
Mauricio Morales *Entomology*
Rodrigo Moran *Entomology*
Carlos Ordoñez *Genetics*
Francisco Ortega, *Physiology/Genetics*
Francisco Rodriguez *Genetics (VVC)*
Luis H Rosero *Pathology*
Sory H Sanchez *Genetics*
Pedro Nel Velez *Genetics*
Daniel Zambrano *Pathology*

* Death during 2001

Annex 2 Donors

Colombia
Ministerio de Agricultura MADR
PRONATTA

France
Agropolis
CIRAD CA
CIRAD Desi
Fondation Aventis Institut de France
Ministry of Foreing Affairs

Germany
BMZ

USA
Rockefeller Foundation
USDA

Venezuela
Centro Tecnologico Polar