Annual Report 1997

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

For Internal Circulation and Discussion Only

November 1997

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Centro Internacional de Agricultura Tropical
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IP-4. Improved Rice Germplasm for Latin America and the Caribbean

Executive Summary

OUTPUT 1. ENHANCED GENE POOLS

Germplasm development

On the identification and utilization of genes from wild germplasm for the improvement of yield and stress resistance, 300 BC2F2 families derived from the crosses Bg-90-2/ *O.rufipogon* and *Caiapo/O. rufipogon,* and 220 families from the cross *O. Llanos 5*/O. *rufipogon* were planted in replicated yield trials in four sites in Colombia (CIAT/Palmira, CIAT-Santa Rosa/Villavicencio, La Libertad Experimental Station/Villavicencio, and Saldaña/Tolima). The *Caiapo/O. rufipogon* cross was planted under upland-savanna conditions, and the other ones under irrigated/ rainfed conditions. In collaboration with EMBRAPA-CNPAF and CIRAD the Caiapo/ *O. rufipogon* cross was also evaluated in Goiania, Goias, Brazil. So far 90 probes out of 140 clones were polymorphic (64%); polymorphism was greater between *O. rufipogon* and the tropical japonica cultivars Fanny, O. Sabana 6 and Caiapo, compared to *O. rufipogon* and the indica cultivars O. Llanos 5 and Bg90-2. Screening of BC2F2 families with polymorphic RFLP clones and microsatellites is underway. Transgressive segregation can be observed, with several lines (11%) having between 5% and 25% higher yield than the recurrent parent Bg90-2. Transgressive segregation for other yield components was also observed. Grain yield data taken on 38 BC2F3 families confirmed results obtained in the BC2F2 generation. These preliminary data is in agreement with reports coming from the work being conducted in China at the Hunan Hybrid Rice Research Center (McCouch, S. 1995) and in South Korea. In summary, preliminary data presented support the hypothesis that DNA introgressed from *O. rufipogon* can contribute positively not only to yield in elite rice cultivars but also in terms of stress resistance

Several countries started to use recurrent selection breeding in 1997: Colombia, Panama, Costa Rica, El Salvador, Venezuela, Argentina, Uruguay and Chile. New populations were locally developed in Argentina, Chile, Uruguay and Venezuela.

Two promising upland rice lines for the Hillsides of the Andes were identified: one from CIAT for the mid-altitude (1300 masl) coffee region and another one from CIRAD-Madagascar for higher altitude (1600 masl), which performed well in field trials in the Cauca region.

Brazil (EMBRAPA/CNPAF) continues to use CIAT/CIRAD upland lines. About 58% of the germplasm in the 1996/97 regional trials comes from CIAT/CIRAD. Half of the parents in their breeding program are also CIAT/CIRAD lines. Identification of new partners for Savanna Upland Rice interested in upland fixed lines: Colombia (Atlantic Coast), Argentina (region of Tucuman), South-Brazil (IRGA), Paraguay and China.

Embryo Rescue and Anther Culture

During 1997, together with the production of doubled haploids for breeding, work also included the rescue of hybrid plants by *in vitro* culture. This activity is designed to aid the recovery of plants from interspecies hybrids between elite irrigated rice, and three wild species (*O. rufipogon, O. barthii,* and *O. glaberrima*).

The laboratory also produced doubled haploids to help IRGA (a FLAR member from Brazil) to fix traits from an advanced promising line (IRGA 959) still segregating for plant type at the F8 generation. Doubled haploids were also produced to advance breeding lines for FLAR. Results indicate that the total percentage of plants selected is similar for doubled haploids and pedigree populations after two cycles of selection at Palmira and Santa Rosa experimental Stations. It is interesting to note, however, that three times as many DH plants were selected at Santa Rosa for disease resistance respect to pedigree, and it was possible to recover lines from three crosses where no lines were derived from pedigree.

OUTPUT 2. KNOWLEDGE OF THE PHYSIOLOGICAL BASIS FOR RICE TRAITS

New Plant Type

In 1997, 446 new plant type selections were evaluated at Santa Rosa. This architecture was designed as an improvement over the standard irrigated dwarf. Among the reported advantages of this plant are improved initial vigor, strong and thick culms, thick dark green foliage, large and full panicles, high harvest index, and low spikelet sterility. In general, initial vigor under our direct seeding conditions was poor. Eighty-six percent of the selections were rated as weak or very weak. Also, 75% were discarded due to high blast disease susceptibility. We selected, however, 31 lines for further testing. These lines were selected for their large panicle size and strong culms. These lines will be included in the germplasm bank and/or distributed to countries.

Submergence Tolerance

We have obtained seeds of reported tolerant genotypes from Asia which we are in the process of evaluating. These lines have many undesirable traits such as weak stems, colored grains, and disease susceptibility. We are hybridizing them with adapted Latin American germplasm. As of 1997 we have made 108 single crosses and have the F1 seed. We are in the process of obtaining markers which are closely linked to the *Sub1* QTL identified by Dr. David Mackill USDA/ARS at the University of California, Davis, as accounting for 69% of the phenotypic variance for the trait in a cross between the tolerant IR40931-26 and susceptible PI543851. First we must confirm that the tolerant germplasm contains the *Sub1* QTL, then use those crosses with the gene to begin the backcrossing program to incorporate it into the adapted varieties.

OUTPUT 3. RICE PESTS AND GENETICS OF RESISTANCE CHARACTERIZED

Blast

Populations of the blast pathogen (234 isolates) collected from the highly resistant cultivar O. Llanos 5 were analyzed for their genetic structure and virulence. These results are leading to understanding pathogen changes in resistance breakdown processes. Blast collections (two distant lineages) since 1989 are being characterized for their genetic structure and virulence to determine pathogen changes overtime. Blast resistance sources to different genetic lineages of the blast pathogen have been identified in the field and greenhouse for genetic crosses by FLAR and the rice project. Fifteen rice lines carrying the complementary (lineage exclusion) resistance genes Pi-1 and Pi-2 were demonstrated to be highly resistant to blast in the field and greenhouse to all genetic lineages of the blast pathogen. Durable blast resistance can be developed following this approach as long as the pathogen is well characterized. Genetic crosses combining complementary resistance sources among Latin American rice cultivars have been developed. Quantitative characterization of the resistance to different genetic lineages of the blast pathogen in 245 recombinant inbred lines using the highly resistant cultivar O. Llanos 5 was generated for the dissection of the resistance genes using molecular markers in collaboration with the Biotechnology Unit.

Rice Hoja Blanca Virus (RHBV) and *Tagosodes oryzicolus*

The development of an improved screening method to select for resistance to planthoppers was accomplished in a collaborative effort between CIAT, IIA-Cuba and FLAR, during 1996-97.

The percentage of lines that are resistant to RHBV is up from 27% in 1995-96 to 44% in 1997, indicating a better incorporation of this trait in the rice gene pools. This is more meaningful when we consider that the capacity of the insect rearing facility was augmented and the amount of germplasm screened also increased. Colombia has identified a promising line with high levels of tolerance to *T. oryzicolus* and RHBV, which will be released next year as a variety. The monitoring of rice fields (done with FEDEARROZ and CORPOICA) reveals that the RHBV level is continuing to increase but at a steady not epidemic rate. For biocontrol *of Tagosodes oryzicolus*, useful entomopathogens were identified and are being tested in the field. To assist in the control of the virus, an early indicator that identifies hotspots before an outbreak of RHBV has been developed.

One single gene controls current resistance in commercial varieties. Therefore broadening the genetic base against RHBV is of great importance. Plant genetic engineer was used to introduce a new gene conferring resistance to RHBV. Various RHBV resistant transgenic lines of the popular variety Cica 8 were produced incorporating the nucleoprotein gene of the virus for cross protection. Biosafety field trials with the most resistant lines, combining agronomic traits similar to the original receptor variety, will follow. Two possible candidate countries for these trials are Costa Rica and Colombia. This work opens for the first time at CIAT the possibility to use bioengineering as a means to introduce agricultural traits for germplasm development.

New resistance sources to hoja blanca and the vector Tagosodes oryziculus were identified in greenhouse and field evaluations.

Other rice diseases

The rice pathogen causing the "helminthosporium" epidemic in 1996 in Colombia was isolated and identified as the well-known species *Bipolaris oryzae*. A different pathogen or new specie did not cause the epidemic. A greenhouse methodology for screening for resistance was developed. A high incidence of the white tip nematode in seed multiplication fields at CIAT was determined. An effective seed treatment to eradicate the nematode from contaminated seed was developed and is being used before seed shipment outside CIAT and to eradicate the nematode from the fields. Several Pytium isolates recovered from infected roots exhibiting the "síndrome de la raíz negra" in Venezuela did not reproduce the symptoms in greenhouse inoculations. The presence of Pytium in the roots is probably a secondary effect of a major soil problem leading to root deterioration.

The transmission of the rice entorchamiento virus (RSNV) by the vector *Polymyxa graminis* was confirmed. A greenhouse method for screening for resistance to the virus was developed. Resistance sources to the virus in traditional and improved cultivars were identified in the greenhouse.

OUTPUT 4. PROJECT PRIORITIES AND RESEARCH CAPACITY ENHANCED

FLAR

In 1997, Guatemala and Paraguay joined the Fund bringing the amount of members to eight countries and three international centers. Contributions reached around US$400,000. In 1998, FLAR will pay 25% of the cost of the Director, whose salary has been fully paid by CIAT until now.

During 1997, FLAR processed more than 300 triple crosses and selected over 4,700 F2 populations for planting in 1998, many of them being crosses made on demand by members. It also has 2,700 R1 anther culture lines originating from 40 triple crosses. The working collection was raised from 330 to 477 parents. More than 11,000 samples from member countries were characterized for grain quality. Other important traits of interest in which work took place include Hoja Blanca Virus, Tagosodes, submergence tolerance, cold and iron toxicity. Another CIRAD scientist (Dr. Michel Valés) joined CIAT/FLAR in August to work in blast and breeding activities. Work with CIRAD and Brazil started for transformation of rice to introduce additional genes for tolerance to iron toxicity.

Farmers' questionnaires

A total of 45 questionnaires applied to a random sample during 1995 in the Tolima (Colombia) rice growing area were analyzed for input use efficiency and gross margins. The results show a wide spread in practices and in gross margins. The top decile (four farmers) had gross margins of

70% over direct costs, while at the other end, the four worst performers showed an average gross margin of 9.5%. As a general rule, the most efficient farmers are more intensive in the use of machinery and rely less on labor and on pesticides. It is difficult to generalize much more than that. The average farm size for the group of the top four farmers is 8.5 has.; the group with the bottom four performers has 20.5 has on average. Seed density is rather high in all cases (over 200 kg/ha) as this is a common strategy against weeds. The main constraints in terms of inefficiencies are found in machinery use (old equipment, frequently rented) and in herbicide use. This points at the high priority that weed management has for these farmers, as weeds are the result of poor preparation, seed densities, herbicide use and many other crop management aspects. For CIAT, this reinforces the need to collaborate in identifying germplasm that can be more competitive with weeds.

Training, Conferences

On the institutional front, FLAR organized three in-country workshops on IPM (Brazil, Costa Rica, and Venezuela) for about one hundred participants in total and a one-month breeders' course in Colombia for twelve participants. Two Brazilian researchers were trained in the new methodologies to characterize blast pathogen populations. Characterization of blast populations from Brazil was initiated during the training at CIAT. A Cuban scientist (J.L. Fuentes) trained in 1996 started his Ph.D. project at CIAT working in the characterization of the genetic diversity of the blast pathogen in Cuba. A student in South Brazil (Renata Cruz Pereira) is doing her PhD thesis with FLAR on the molecular characterization of cold tolerance. A Colombian student (R.D. Zárate) will start field work in 1998 on blast. Another Colombian, Hernando Ramírez is doing its dissertation on rice transformation. A student from the U.S., Kenneth Seebold, finished his dissertation on the use of silicon for the control of rice diseases and yield improvement on acid soils. Four Master of Science students (3 from Colombia—Edgar Corredor, Iván Lozano and Luis E. Berrío--, and one from Venezuela –Carlos Gamboa--) are conducting thesis work at CIAT. Five scientists are under the scholarship agreement with Colciencias. Twelve scientists received in-house training during 1997 (anther culture, breeding, pathology, entomology, virology).

A highlight of the year was the celebration last March in Venezuela, of the X INGER-LAC Conference (close to three hundred participants), a workshop for breeders, and the Fifth World Rice Day co-organized with IRRI and FUNDARROZ and inaugurated by the President, Dr. Rafael Caldera. Close to 100,000 (one hundred thousand) persons attended this Rice Festival in Caracas.

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

Introduction and Overview

Rice research in Latin America and the Caribbean (LAC) has emphasized growth, equity and the enhancement of the resource base. Rice is particularly important from these standpoints. Technological progress, improved efficiency, marked production increases and important linkages with the rest of the economy have put rice at the top of priorities for agricultural growth policies in most countries of LAC. Rice is preferred by the poor because it is cheap, nutritious, appealing, easy to prepare, and easy to store and transport. Rice has also been a pioneer crop in Latin America playing a protagonist role in the agricultural frontier of the lowlands, the savannas and the forest margins. More recently, promising lines for the hillsides have been developed, in collaboration with CIRAD, to bring this important food staple into the range of food crop alternatives for poor farmers in this ecosystem. Eighty percent of the growth in rice production (at the annual rate of 2.5%) in the 1967-97 period, was due to growth in productivity, releasing the pressure on land expansion in the quest for additional food.

Besides outstanding technical innovation, two major "external" reasons why rice research has had greater impact than any other area of work at CIAT are: i) simplicity of seed-borne technology delivery, for a crop that is as simple and inexpensive to multiply as rice; and ii) the well-organized commercial rice sector in LAC, which quickly adopts new technology. These external factors will continue to give rice research a comparative advantage in achieving future impact.

Research at CIAT's Rice Project is executed in the framework of activities leading to achieve four main outputs:

Output 1. Gene Pools Enhanced

Output 2. Knowledge of the physiological basis for rice traits enhanced

Output 3. Rice Pests and Genetics of Resistance Characterized

Output 4. Project Priorities and Research Capacity Enhanced

The future of rice research holds exciting challenges and opportunities. Rice research can continue to make significant contributions to environmental goals such as the protection of rain forests and reduction of agrochemical use, as well as in feeding people. The progressive involvement of the private sector in funding national and regional rice research through FLAR shows that this sector continues to be at the forefront of technology development and institutional maturity in LAC countries.

OUTPUT 1. ENHANCED GENE POOLS

New varieties represent a "pooling" of valuable new traits into an adapted genetic background that farmers can use to increase and stabilize yields. By improving production efficiency, these varieties generate cost savings to farmers, much of which is passed on to consumers in the form of lower prices.

It has been estimated using pedigree information, that the relative diversity of the genetic core of LAC irrigated rice is already reaching its limits in terms of yield potential. Further yield enhancement requires the design of alternative genetic combinations. Monitoring advances in genetic base diversification may benefit from methodologies more precise than pedigree analysis. CIAT's major research in rice germplasm development is typically focussing in pre-breeding activities with the aid of molecular markers: testing and adaptation of the new plant types, commercial crosses with wild rices, population improvement as well as some conventional pedigree breeding work done in collaboration with national rice programs. CIAT's new role in the 1990's is to complement breeding efforts of NARS and other partners in the region (see **Output 4.** below). Promising lines are made available to other NARS through the INGER network, and serve as parents for further regional breeding use. The objective is to develop high-yielding germplasm adapted to irrigated, rainfed lowland and upland conditions, tolerant to major diseases and insect pests, with good grain quality, and early to intermediate growth duration. To ensure good disease pressure, "hot spot" sites are used.

Ongoing plant improvement efforts must be streamlined using a mixture of biotechnological and classical approaches. The main objective of the activity on the identification and utilization of genes from wild germplasm for the improvement of yield and stress resistance is to develop and implement a marker assisted breeding strategy that will lead to improved cultivars and simultaneously broaden the genetic base of cultivated rice.

The rice project emphasizes the enhancement of populations while has slowed down considerably the production of fixed lines for direct release by NARS in the region. Part of the strategy is to develop and enhance gene pools and populations for well-targeted trait(s) to be used as source of potential parents by the regional breeding programs. One suitable breeding method to achieve this goal is Recurrent Selection. In 1992, the CIRAD/CIAT rice improvement collaborative project introduced from Brazil and French Guyana, gene pools and populations segregating for a male-sterile recessive gene. The main objectives of the project are:

- To understand the performance of the introduced germplasm in the upland acid soils of the colombian savannas,
- To maintain the germplasm by harvesting fecundated male-sterile plants,
- To identify adapted fertile genotypes for use in breeding programs for fixed lines,
- To start recurrent selection by recombining the best selected genotypes in the introduced germplasm, and
- To create new populations by incorporating the best locally adapted lines of CIAT upland rice breeding program into the best adapted introduced germplasm that provides a good source of male-sterile background.

Wetland Rice

More efficient rice varieties with higher, more stable yields are the essence of what happened in the lowland rice "Green Revolution". New semidwarf varieties enabled farmers to achieve a 45% yield increase across LAC's the most favored irrigated rice area from 3.5 mt/ha in 1966 to 5.5 MT/ha in 1997, stimulating an increase in production efficiency and an expansion of irrigated rice production from 4.3 million MT to 14.5 million MT over that period. Wetland rice production reached 17.0 million tons by 1997, or over 80% of rice production in LAC.

The ongoing nature of this impact is reflected in the rather steady rate of release of new wetland varieties, since the start of the Green Revolution. New varietal releases are an important indicator of progress, because they usually represent improvement for at least one key trait, while maintaining other gains already achieved. Over the past 25 years there have been an average of 10 new lowland varieties released per year across LAC. A number of new traits are being generated in Latin America, as well as globally, which promise to continue the remarkable record of past impact.

Market-acceptable grain quality is important for LAC rice varieties. Most Latin American rice consumers prefers long, slender, translucent grains that cook dry and loose, and remain soft after cooling. High percentage of head rice is required by millers. The objective of this activity is to better understand the genetic control of factors affecting grain quality. On the other hand, demand for alternative uses of rice is increasing, particularly in the U.S. Research on new uses of rice appears as an important window of opportunity for the next century in Latin America.

Anther culture to bridge wide crosses is now a routine and useful tool in breeding in this part of the world. Our experience shows that anther culture can be used to overcome sterility in wide crosses. It also appears to produce more "intermediate" types of recombinants, as opposed to the "parental" types that seem to emerge from wide-cross populations when generations are advanced through selfing. Since 1985 CIAT has incorporated anther culture (AC) as a tool to reduce generation time in rice. Research on AC has increased the yield of doubled haploids, making the tool more cost-efficient for breeders. When compared with the traditional pedigree method, AC appears to be an economically practical new tool. Several national programs in LAC have started to apply the AC protocol.

Upland Rice

Much higher-yielding savanna upland rice germplasm developed in the 1980's is now reaching the farm and promises an important breakthrough for the savannas. The new upland varieties are shorter, have a more efficient use of fertilizers, do not lodge so easily and have better grain quality than traditional upland varieties.

About 40% of Latin America's 6.7 million hectares of rice are in the freely drained (aerobic-soil) uplands, producing almost 20% of LAC's rice crop. About two-thirds of the total upland area are in the savannas of Brazil where large, mechanized farmers predominate. Upland rice is more tolerant of the acid soils of these areas than any other major food crop, so it plays a strategic role in the development of sustainable systems there. Traditional upland rice farming accounts for about 5% of the regional production of rice (or about 1.0 million MT). Yet, over 80% of rice farmers in LAC belong to this system. These farmers are rather poor and constitute important niches where rice improvements can have significant impact on their livelihood. However, adoption of modern varieties by those groups is relatively low (less than 30%).

Rice is a major staple of forest-margin and hillsides smallholders, many of whom live in extreme poverty. The new upland rice germplasm being developed for hillside conditions (with tolerance to mid-altitude climatic conditions) promises to add this important cereal into the range of current cropping alternatives, enhancing their production and nutrition as well. The new upland rices will help drive the adoption of improved pastures, a leverage effect far beyond the value of the rice crop itself. These "agropastoral systems" could provide the region with a viable alternative to sustainable approaches for settlers in the Amazon forests that need to satisfy basic food needs.

Since breeding for this agro-ecosystem is fairly new in most LAC countries other than Brazil, much of the effort in this research area goes to developing and strengthening linkages with national as well as international groups, including planning of joint projects, exchange of germplasm and methodologies, training, and collaborative research. CIAT, CIRAD and CNPAF (in Brazil) are members of the Upland Rice Research Consortium, coordinated by IRRI.

OUTPUT 2. KNOWLEDGE OF THE PHYSIOLOGICAL BASIS FOR RICE TRAITS GAINED

The main subactivities designed to obtain this output have been the work with IRRI's new plant type, the search for components for more efficient weed control and the study of mechanisms for tolerance to low Phosphorus and acid soils.

With the departure of the JIRCAS scientist in early 1997, his five year work on mechanisms for tolerance to low Phosphorus and acid soils was finished. Furthermore, budget adjustments precluded the incorporation of a postdoctoral scientist to continue the agronomy/physiology work in 1997. However, the project has been able to maintain strategic work with the new plant type and in the identification of more competitive rice germplasm for more efficient weed control.

In 1997, 446 new plant type selections were evaluated at Santa Rosa. This promising material constitutes a new source of germplasm that will contribute to broaden the genetic base and should contribute to break yield ceiling in the near future. Its architecture was designed as an improvement over the standard irrigated dwarf. Among the reported advantages of this plant are improved initial vigor, strong and thick culms, thick dark green foliage, large and full panicles, high harvest index, and low spikelet sterility. In general, initial vigor under our direct seeding conditions was poor. Eighty-six percent of the selections were rated as weak or very weak. Also, 75% were discarded due to high blast disease susceptibility. We selected, however, 31 lines for further testing. These lines were selected for their large panicle size and strong culms. These lines will be included in the germplasm bank and/or distributed to countries.

The work on components for weed control focused on identifying germplasm with allelopathy and anaerobic vigor. The greatest expenditure in pesticides at the regional level ($218 million, or 45% of the total) is on herbicides. IPM strategies involving thresholds, rotation, better water control and seeding practices, and use of varieties with weed-interference properties and adapted to water seeding could probably reduce herbicide use by at least half. Weeds are the number one pest of rice throughout LAC. In Colombia and Venezuela, unpublished panel data for 1991 to 1996 from producers show that the weed control represents an increasing share of crop production costs and has scalated from around 12% to 15%. Chronic annual production losses of 11% are estimated, as compared to 7% for diseases and 4% for insects.

Weed scientists report that very few new herbicides are coming into the market because of the high costs of registration due to environmental concerns.

While tillage and herbicides will continue to play a major role in the future, there is clearly a need for complementary approaches, which are cost-effective and environmentally friendly. IRRI, WARDA, CIRAD and CIAT have recently begun research programs on traits of rice to enhance weed control in their agro-ecosystems. A collaborative effort between CIRAD, CIAT and FLAR with NARS in the region is identifying and characterizing the rice weeds in Latin America and the Caribbean to produce a CD-ROM that will be of great value to extension workers, farmers, reserchers and students in the region. The program is based on an existing version developed by CIRAD for cotton in Africa (called ADVENTROP).

We estimate that rice traits for enhancing weed control could probably reduce weed control costs by 30%. This would more than justify the research investment.

OUTPUT 3. RICE PESTS AND GENETICS OF RESISTANCE CHARACTERIZED

Durable Blast Resistance

Blast is one of the most intractable fungal pest problems in cereal cultivation. Crop losses cost an estimated US$200 million annually. When resistance is not effective, farmers use fungicide sprays. This costs the region an estimated $170 million annually, or 35% of total pesticide expenditures. Besides being costly, most are hazardous both to the applicator and the environment. Durably-resistant varieties, lower seeding rates, more efficient use of lower amounts of applied nitrogen fertilizer, the use of silicon fertilizers, and fewer but better-timed fungicide applications cut the need for the use of these chemicals by more than half.

High levels of genetic resistance exist, but they are ephemeral. The fungus rapidly overcomes resistance. "Durable" resistance is a concept receiving much theoretical attention, but progress has been difficult and slow. One of the world's most blast resistant varieties, Oryzica Llanos 5, is a recent product of CIAT's gene-pyramiding approach. There are over 10 advanced lines in this region that have a similar level of resistance to Oryzica Llanos 5.

The technique called MGR-DNA fingerprinting allows to reveal the genetic structure of blast in a way not previously possible. This technique can act as an "early warning system" to detect the appearance of new genetic lineages of blast. It might also help breeders identify resistance genes that are more durable than in the past.

The main advances during 1997 were:

1. Understanding dynamics of the blast pathogen in time and pathogen changes leading to resistance breakdown

2. Identification of sources of resistance to blast for combining complementary resistance genes

3. Development of rice lines (F6) combining complementary blast resistance sources that exclude pathogen population in Colombia

4. Training of national and international scientists in new rice blast research as well as providing guidance for the development of blast research projects in different Latin American countries

Diversified Tagosodes/Hoja Blanca Resistance

The most hazardous pesticides are the insecticides, which account for an estimated $95 million, or 20% of the region's total pesticide bill. Control of the *Tagosodes* leafhopper by pesticide application is often self-defeating because it eliminates the predators that could assist in keeping this pest in check. Practical methods for monitoring the predator population as well as that of pests could give farmers the confidence to avoid unnecessary sprays.

Rice hoja blanca virus (RHBV) causes severe recurrent epidemics and is exclusive of the Andean, Central American and Caribbean countries of tropical LAC. It is transmitted by the planthopper insect *Tagosodes oryzicola*, which can also cause serious feeding damage even when not viruliferous. Colombian rice farmers were spraying up to 5-6 times to control the RHBV vector and other insect pests in the 1980's. The uncertainty of epidemics induces farmers to spray even when the problem is not apparent, as "insurance". This costs the region about US$15 million annually.

The RHBV-Tagosodes problem provided the original impetus for creation of the Rockefeller-funded rice improvement project at ICA (Colombia) in the 1950's, the predecessor of CIAT's Rice Program, started in 1967. It also represents one of its most notable successes: Tagosodes resistance was achieved by 1970 (CICA 4 variety) and was increased in CICA 8 (1978), based on the "Tetep" varietal source.

RHBV resistance based on the "Colombia 1" source was added by 1989 (Oryzica Llanos 4). There is risk, however in depending on only a single resistance source for each pest for the whole region. New sources have been identified but their genetic control and crossability to adapted LAC materials need to be understood and applied. Transformation with viral genes is also being attempted to create an entirely novel resistance source.

This is an integrated pest management project that has had a broad range of activities. The strategy to enhance germplasm pools with resistance againts RHBV and *Tagosodes oryzicolus* continues to show improvement as the rate of lines selected for RHBV went from 27% to 44% in 1997 and more lines were evaluated. Knowledge of the insect and spread of the disease is another basic activity that is taking palce in Colombia and is starting to be done in Venezuela. other countries will have to follow. The strategy for control complements the varietal approach with the use of agrochemicals and identification of biological control alternatives. Training plays key role. During 1997, there were three in-country courses on IPM for about one hundred participants.

For Tagosodes in breeding populations, a new screening method was developed by CIAT, el IIA-Cuba y el FLAR during 1996-97. It tests segregating populations and advanced rice breeding lines for resistance to non-viruliferous Tagosodes (resistance to feeding damage per se). Potential parents for crossing as well as characterized lines are sent to NARS. For resistance to Rice Hoja Blanca Virus (RHBV) in breeding populations, the screening activity tests segregating populations and advanced rice breeding lines for RHBV behavior. These activities are strengthened with the production of transgenic rice with resistance to RHBV. Various RHBV resistant transgenic lines of the popular variety Cica 8 were produced incorporating the nucleoprotein gene of the virus for cross protection. Biosafety field trials with the most resistant lines, combining agronomic traits similar to the original receptor variety, will follow.

"Entorchamiento" virus

This virus was reported in Africa in 1977 and was detected in Colombia in 1991. A major research achievement in this area during 1997 was the confirmation of the transmission of the rice entorchamiento virus by *Polymyxa graminis* and the identification of genetic resistance to the virus.

OUTPUT 4. PROJECT PRIORITIES AND RESEARCH CAPACITY ENHANCED

The past three decades have resulted in strong national rice improvement programs, high-yielding rice varieties on farmers' fields, and networks of germplasm improvement and related information linked, via CIAT, to the premier upstream research resource, IRRI. In the 1990's, CIAT's rice project evolved from the breeding and agronomy approach of the past into an strategy focusing on pre-breeding activities that complement the work of the new partnership that emerged in 1995: the Fund for Latin America and the Caribbean Irrigated Rice (FLAR).

FLAR

Building on the successful model of the past three decades and on the stock of capital for sustained progress while assuring its continued dedication to the tasks ahead is the challenge for the rice sector of LAC and the main purpose of the Irrigated Rice Fund for Latin America and the Caribbean (FLAR). The Fund, created in 1995, brings resources from private and public national organizations. It assumed part of the responsibility and the control of the regional rice research agenda. FLAR appears to be a viable alternative by reason of several emerging constraints and opportunities.

In 1997, Guatemala and Paraguay joined the Fund bringing the amount of members to eight countries and three international centers. Contributions reached around US$400,000. In 1998, FLAR will pay 25% of the cost of the Director, whose salary has been fully paid by CIAT until now.

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On the institutional front, FLAR organized three in-country workshops on IPM (Brazil, Costa Rica, and Venezuela) and a one-month breeders' course in Colombia. A highlight of the year was the celebration last March in Venezuela, of the X INGER-LAC Conference and the Fifth World Rice Day co-organized with IRRI and FUNDARROZ and inaugurated by the President, Dr. Rafael Caldera.

Farmers' questionnaires, rice technologies and costs

The Rice Project has collaborated with FEDEARROZ (Colombia) and FUNDARROZ (Venezuela) in the design and applications of farmers' questionnaires since 1989. During 1997, data collected from 45 farmers in a 1995 survey in the Tolima (Colombia) region were analyzed. The results show a wide spread in practices and in gross margins. The top decile (four farmers) had gross margins of 70% over the direct costs, while at the other end, the worst four performers showed an average gross margin of 9.5%. As a general rule, the most efficient farmers are more intensive in the use of machinery and rely less on labor and on pesticides. It is difficult to generalize much more than that. The average farm size for the top four producers is 8.5 has. while the bottom four producers have 20.5 has on average. Seed density is rather high in all cases (over 200 kg/ha) as this is a common strategy against weeds.

The main constraints in terms of inefficiencies are found in machinery use (old equipment, frequently rented) and in herbicide use. This points at the high priority that the weed management aspect has for these farmers, as weeds are the result of poor preparation, seed densities, herbicide use and many other crop management aspects. For CIAT, this points out at the need to collaborate in identifying germplasm that can be more competitive with weeds. For 1998, we will analyze sets of data from Venezuela (from the two major growing areas in Guárico and in Portuguesa) as well as new data from Colombia.

The current report presents a detailed account of the main activities conducted to achieve the four outputs that were targeted for 1997 and that will continue to be our goals into the next three years as they form part of CIAT's Medium Term Plan, 1998-2000.

Luis R. Sanint
Project Manager, IP-4
Director of FLAR

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

Project Goal

To improve the nutritional and economic well-being of rice growers and low income consumers in Latin America and the Caribbean through sustainable increases in rice production and productivity

Project Purpose

To increase genetic diversity and enhance gene pools for higher, more stable yields with lower unit production costs and reduce environmental hazards

Outputs

Enhanced Gene Pools

Activities

1. Introduce germplasm from different sources.
2. Identify progenitors for crossing.
3. Generate and evaluate gene pools.
4. *Seed multiplication and* distribution to FLAR and other partners.
5. Identify useful traits in wild germplasm and make interspecific crosses (linked to Project SB-2).
6. Use AC/embryo rescue to speed up development of breeding lines.
7. Select for useful traits, with the aid of molecular markers (linked to Project SB-2).

Knowledge of the physiological basis for rice traits

1. Characterize and promote new plant type for direct seeding.
2. Improve demand-driven N supply and N uptake *for full* expression of yield potential.
3. Understanding the physiological mechanism for tolerance to low P and acid soils.
4. Make crosses of competitive cultivars with submergence tolerant, and early-vigorous progenitors.
5. Conduct close response tests and field evaluation for diagnosis of herbicide resistance and cross resistance, and to monitor management practices.

Rice pests and genetics of resistance characterized

1. Monitoring genetic and virulence diversity of blast pathogen.
2. Improve breeding methods for developing durable blast resistance.
3. Evaluation of rice germplasm including transgenic rice for resistance to *Tagosodes orizicolus and RHBV.*
4. Collaboration with FEDEARROZ to transfer Tagosodes and RHBV *evaluation technics.* Conduct field surveys.
5. Isolation and characterization of the causal agent and vector of "entorchamiento".
6. Development of germplasm screening technique for resistance and implementation of "entorchamiento" control measures

Project priorities and research capacity enhanced

1. Coordinate breeding activities with FLAR/other institutions (linked to Project SN-2).
2. Organize/support training and information activities (linked to Project SN-2).
3. Characterize rice technologies and costs (irrigated and upland).
4. Apply questionnairies to *farmers with national* programs.
5. Refine priorities.
6. *Training national scientists of* Latin America in new research technologies.

LOGICAL FRAMEWORK - PROJECT IP-4

Project Title: Improved Rice Germplasm for Latin America and the Caribbean   Project Manager: Luis R. Sanint

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
Goal To improve the nutritional and economic well-being of rice growers and low income consumers in Latin America and the Caribbean through sustainable increases in rice production and productivity.	Improved access of rice growers and consumers to standard goods and services. Reduction in pesticide use and increase yield average/ha. Increase in the number of ha planted with new cultivars.	National statistics on agriculture and development of LAC. Rice production statistics.	Donors, governments and NARS continued interest in sustainable increase in rice production.
Purpose To increase genetic diversity and enhance gene pools for higher, more stable yields with lower unit production costs of rice and reduce environmental hazards.	Evaluations of yield potential of F2BC2, end 1997. Increased use of improved populations from recurrent selection by NARS at the end of 1997. Rice lines selected with desired gene traits. Potential donors high levels of blast resistance.	Database on seed exchange. Project, CIAT and NARS annual reports.	Improved/diversed populations are adopted/used by NARS; policies favor adoption. Farmers are willing to reduce pesticide use.
Output 1 Enhanced Gene Pools.	Seed of best gene pools distributed to FLAR and 50% of other partners by the end of 1997.	Project progress report for 1997.	Continued demand for these populations. NARS willing to try out/use improved lines.
Activities - Introduce, identify, generate and evaluate germplasm from different sources. Multiply seed to FLAR/ other partners. Use AC/ embryo rescue (CM, MCH, ZL, P-Docs). - Identify and select for useful traits with the aid of molecular markers (linked to Project SB-2) (JT, FC, CM).	First evaluation of the yield potential of 3 F2BC2 populations conducted by 1997 in 3 sites. Number of field trials planted. Number of crosses made, DH obtained, hybrid plants recovered by embryo rescue and traits identified. QTLs identified.	Project progress report for 1997. Field visits to testing sites. Budget.	Adequate funding and timely release of budget. Continued support from CIRAD-CA. Useful traits in wild germplasm can be incorporated in improved populations. NARS willing and capable to try out/use new improved populations.
Output 2A Knowledge of the physiological basis for yield enhancement and adaptation to acid soils.	Main 5 agronomic/physiological traits measured beginning 1997.	Project progress report for 1997. Two publications.	Continued demand by NARS for these populations and knowledge.
Activities - Characterize new plant type under direct seeding and N uptake. Introgress new plant type into LAC's gene pools (Post-Docs, CM, HR) - Understand the physiological mechanisms for tolerance to low P and acid soils (P.-Doc)	N-management for new plant type worked out by the end of 1997. First BC to new plant type made by end 1997. Mechanism for tolerance to low P/acid soils proposed.	Project progress report for 1997.	Continued JIRCAS interest and support. Adequate funding and timely released of budget. Rice support staff in plant physiology in place. Post-Doc in place.
Output 2B Weed control enhanced by the use of new genotypes and practices.	Weed competitive varieties developed. Herbicide - resistant rice cultivars to control red rice and herbicide resistant weeds developed.	Project reports.	Farmers adopt technology developed. Germplasm adoption is higher and more consistent than of agronomic changes.
Activities - Screen for competitiveness and assess productivity. Make crosses of competitive cultivars with submergence tolerant, and early-vigorous progenitors (P-Doc, CM) - Conduct tests for herbicide and cross resistance, and monitor management practices. Evaluate herbicide resistant transgenic and non-transgenic plants (Post-Doc, ZL, HR linked to project SB-2)	Transgenic plants. Identified traits for competitiveness.	Workplan. Budget.	CIAT-RP representative will continue to participate in COMALFI herbicide resistance committee, and will link with similar efforts in LAC. Post-doc in weed agronomy in place.

Output 3A Rice blast pathogen and genetics of resistance characterized.	Isolates characterized for their virulence and genetic structure.	Project progress reports.	Collection of blast infected samples gives viable isolates. Molecular markers available from BRU.
Activities - Monitoring genetic and virulence diversity of pathogen. Testing breeding methods for durable blast resistance (CM, FC) - Dissecting blast resistance genes in highly resistant cultivars and make new crosses (CM, FC, linked to project SB-2)	Isolates.	Assigned budget.	Rice crosses and populations developed by rice breeders. Biotechnology unit continues identifying molecular markers associated with resistance.
Output 3B Rice lines with diverse resistance to *Tagosodes* and RHBV developed.	RHBV and vector surveys implemented. Evaluation and biocontrol methods developed.	Project reports.	Existence of diversified resistance to RHBV and Tagosodes.
Activities - Evaluation of rice germplasm including transgenic plants for resistance to *T. orizicolus* and to RHBV (CM, FC, ZL) - *Studies of RHBV colony vs date vs variety* (LC, CM) - RHBV surveys in rice fields and epidemiological studies. Biocontrol of *T. orizicolus* (LC, CM, FC) - Collaboration with NARS to transfer evaluation technics (LC, CM, FC)	Rice lines with diversified resistance to *Tagosodes orizicolus* and to RHBV. More effective colony management. Baseline information for understanding and prediction RHBV epidemics, crop management. Increased capacity of NARS to screen germplasm. Effective entomopathogens for insect control. Transgenic lines with RHBV-viral genes w/reduced disease symptoms.	Workplan. Budget plan.	Collaboration with FEDEARROZ, FLAR. Depends partially on special project funding.
Output 3C The causal agent of the "entorchamiento" problem characterized.	Identification and characterization of causal agent.	Project reports.	Recommendations issued and adopted by farmers.
Activities - Isolation and characterization of the causal agent and vector of "entorchamiento" (FC, FM). - Development of diagnostic methods and germplasm screening technique to implement control measures (FC, FM).	The causal agent of the "entorchamiento" disease of rice and its vector are characterized, managed. Different control strategies are implemented.	*Publications and* diagnostic kits available. Resistant germplasm selected under artificial conditions. Workplan, budget.	*Available infected material can be* maintained and propagated by artificial measures. Recommendations issued and adopted by farmers. Special funding: COLCIENCIAS and ODA
Output 4 Priorities and research capacity enhanced	One workshop conducted by 1997 for NARS. 15-20 trained people from NARS. Farmers' surveys in LAC.	Project progress and workshop report for 1997.	NARS continued interest in specialized training and information exchange. Linkages with NARS.
Activities - Coordinate research, training activities with NARS. Establish priorities (LS, MCh, FC, ZL) - Apply questionnaires to rice farmers (LS).	*Research plans written. Number of scientists trained.* Costs of production, production coefficients Budget.	Progress report for 1997.	Adequate funding and timely released of budget.

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

Synopsis

Objectives: To increase genetic diversity and enhance gene pools for higher, more stable yields with lower unit production costs of rice; and reduce environmental hazards.

Outputs: Improved rice population with a wider genetic diversity that can be used as progenitors to obtain: enhanced gene pools and knowledge of the physiological basis for rice traits. More rational pesticide use. Rice priorities and research capacity enhanced.

Gains: Broader genetic base available and germplasm better characterized for the use by National Programs and other rice research institutions. New sources of resistance to diseases, viruses and insects incorporated and available to partners. Higher yielding advanced rice lines. Information on the variability and stability of progenitors and of advance materials available to increase breeding efforts. Rational pesticide use with lower environmental hazards. Lower unit costs conducive to higher profits and lower rice prices to consumers.

Milestones:

1998 Wild *Oryza spp.* backcrosses with commercial varieties advanced to F3. New plant type characterization and crosses for LAC made. Population improvement for irrigated rice by recurrent selection. Patterns of economic efficiency in rice production in Colombia identified. New methodology for screening and *Tagosodes* spp. and "hoja blanca virus" (HBV) resistance.

1999 Use of wild germplasm genes: transgressive QTLs identified. New plant type as progenitors. Resistance to "entorchamiento" (twisting) identified. Stable integration of the ph gene for HBV resistance. Blast lineages from temperate zone characterized and resistant donors selected.

2000 Enhanced gene pools available from wild crosses, recurrent selection and new plant type, for testing and use. Transgressive QTLs used as basis for selecting new lines.

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

Collaborators: FLAR (the Latin American and the Caribbean Fund for Irrigated Rice), WARDA, NARS (e.g. EMBRAPA, CORPOICA, FONAIAP, IDIAP, INIAP, INIA, IIA, etc.) U.S. Universities (Cornell, Purdue, L.S.U., Arkansas, Texas A&M, California, Florida State). IRRI, CIRAD, JIRCAS, WARDA. Seed companies from private sector.

CGIAR system linkages: Breeding (60%); Crop systems (5%); Protecting the Environment (5%); Saving Biodiversity (20%); Strengthening NARS (5%); Improving Policies (5%). Linked to IRRI global rice research.

CIAT project linkages: New methods from SB-1 and SB-2. Provide improved germplasm to PE-1, PE-2, and PE-3.

OUTPUT 1. ENHANCED GENE POOLS

A. IDENTIFICATION AND UTILIZATION OF GENES FROM WILD GERMPLASM FOR THE IMPROVEMENT OF YIELD AND STRESS RESISTANCE.

C. Martínez, J. Lopez, J. Borrero. A. Almeida and J. Tohme

Collaborators:

S. McCouch, Dept Plant Breeding, Cornell University
F. Correa, CIAT Rice project
M. Chatel, CIRAD-CIAT, Rice project
E. Guimaraes, CNPAF, Goiania, Brazil

INTRODUCTION

Rice forms the nutritional basis for much of the word's populations; it is planted on 148 million hectares worldwide and is considered the world's most important food crop; globally rice provides 20% of energy and 15% of per capita protein. More than 90% of the word's rice is grown and consumed in Asia, while Latin America's rice production represents 3.5% of the total. Rice production in Latin America increased from 9.9 to 18.8 million tons of paddy rice from 1966 to 1995 due mainly to the use of improved varieties and crop management.

However, rapid population growth in Asia and Latin America is putting increasing pressure on the already strained food-producing resources of these regions. Besides, 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-pollinated crops, like rice (Wang et al. 1992). This reduced genetic variation renders modern crop varieties more vulnerable to biotic and abiotic stresses, and could explained the already observed slower rate of genetic progress achived by plant breeders (Tanksley and Nelson, 1996). There is an urgent need to increase rice production in a sustainable manner. New paradigms of international, regional and interinstitutional cooperation and new strategies for crop improvement are needed. Ongoing plant improvement efforts must be streamlined using a mixture of biotechnological and classical approaches. The main objective of this project is to develop and implement a marker assisted breeding strategy that will lead to the development of improved cultivares and simultaneously broaden the genetic base of cultivated rice.

MATERIALS AND METHODS

Population development

Few plants (2-3) in each of the wild species *O. rufipogon, O. glaberrima,* and *O. barthii* were hybridized to several plants of each of the improved rice cultivars (recurrent parent) listed in Table 1.

Table 1. Plant materials used in interspecific hybridization at CIAT

Parent	Accesion No.	Source	Origin	Notes
Donor				
O. rufipogon	105491	IRRI	Malaysia	Ancestor of O. sativa
O. glaberrima	103544	IRRI	Mali	Cultivated in Africa
O. barthii	104119	IRRI	Chad	Relative of O.glaberrima
Recipient (Recurrent parent)				
Cypress		Louisiana	USA	Tropical japonica, quality
Lemont		Texas	USA	Tropical japonica, quality
RU9403006 (Jefferson)		Texas	USA	Tropical japonica, quality
Oryzica Llanos 5		CIAT	Colombia	Indica; resistant to P. Oryzae
BG90-2		CIAT	Sri Lanka	Indica; high yield
Morelos A88		CIAT	Mexico	Good combining ability
Oryzica 3		CIAT	Colombia	Indica; high yield
O. Sabana 6		CIAT	Colombia	Tropical japonica; upland
O. Turipana 7		CIAT	Colombia	Tropical japonica; upland
Progresso		CIAT	Brasil	Tropical japonica; upland
CAIAPO		CIAT	Brasil	Tropical japonica; upland
CT6196-33-11-1-3		CIAT	Colombia	Tropical japonica; upland

Recurrent parents were choosen based on specific agronomic traits such as yield potential, grain quality, disease resistance, etc. Single crosses were obtained and grown in the greenhouse at CIAT in early 1994. Three F1 hybrid plants were backcrossed to the improved cultivar, using the latter one as the female parent; approximately 100-180 BC1F1 seeds were obtained per cross combination. The resulting BC1F1 plants were transplanted (30x50 cm) and evaluated based on phenotype; negative phenotypic selection for undesirable agronomic traits (spreading plant type, excessive shattering, long awns, dark-color grains, high sterility, etc) was used to narrow the selection down to the best (40-50) individuals. Each selected BC1 individual was back crossed again to the recurrent parent and approximately 30 BC2F1 seeds were produced; 20 BC2 seeds from each of the selected BC1 plant were sown in wooden trays in the screehouse and later on transplanted (30x40 cm) under irrigated conditions. A negative phenotypic selection was applied again and best individuals per cross were selected and harvested individually to generate BC2F2 seed; approximately 220-300 BC2F1 plants were selected per cross combination for field testing. Each selected BC2F1 plant was evaluated for 12 agronomic traits including days to heading and maturity, plant height, panicle length, panicles per plant, spikelests per panicle, grains per panicle, seed set rate, spikelests per plant, grains per plant, 1000-grain weight and yield per plant. Based on field observations and genetic potential only three populations (Bg90-2/ *O. rufipogon*, *O. Llanos 5*/ *O. rufipogon*, and *Caiapo/O. rufipogon* were chosen for field testing.

Field trials (BC_2F_2 and BC_2F_3 families)
The 300 BC2F2 families derived from the crosses Bg-90-2/ *O.rufipogon* and *Caiapo/O. rufipogon,* and the 220 families from the cross *O. Llanos 5/*O. *rufipogon* were planted in replicated yield trials in four sites in Colombia (CIAT-Palmira and CIAT-Santa Rosa, Villavicencio, La Libertad Exp. Station, Villavicencio, and Saldaña. Tolima). The *Caiapo/O. rufipogon* cross was planted under upland-savanna conditions, and the other ones under irrigated/rainfed conditions. In collaboration with EMBRAPA/CNPAF/CIRAD the Caiapo/O. *rufipogon* cross was also evaluated in Goiania, Goias, Brazil. Transplanting (20x30cm) was used at CIAT-Palmira, while direct seeding was used elsewhere. A completely randomized design with two reps., 2 row-plot, 5 m. long. was used. Data on the 12 agronomic data described elsewhere, including plot yield/family were taken on 10 randomly selected plants/plot.
Based on yield potential and good agronomic traits, 38 BC2F2 families from the cross Bg90-2/O. rufipogon were selected and further evaluated for grain yield; a completely randomized design with four reps, 4 row-plot, and 5m. long was used.

Molecular characterization
Parental surveys filters containing *O. rufipogon,* Fanny, O. Llanos 5, Bg90-2, Caiapo and, O. Sabana 6, and the corresponding F1's were prepared using five restriction enzymes (Eco RI, EcoRV, Hind III, and Dra I). Approximately 140 markers from the rice molecular framework linkage map were selected at 10-20 CM intervals throughout the genome. A set of 50 mapped rice microsatellite markers, which were developed at Cornell University, is also being used to complement the RFLPs in QTL analysis. DNA from each BC2F2 family of the Bg90-2/ *O. rufipogon* cross has already been extracted.

Results and Discussion

Probe selection
Data indicated that so far 90 probes out of 140 clones were polymorphic (64%); polymorphism was greater between *O. rufipogon* and the tropical japonica cultivars Fanny, O. Sabana 6 and Caiapo, compared to *O. rufipogon* and the indica cultivars *O. Llanos* 5 and Bg90-2. Screening of BC2F2 families with polymorphic RFLP clones and microsatellites is underway.

Population development
A total of 36 crosses was made using the parental lines listed in Table 1; population development from these crosses is underway but at different stages; the more advanced populations have gone through two-three rounds of backcrossing to the recurrent parent. Several populations involving crosses between Lemont, Cippress and Jefferson, *with O. rufipogon, O. barthii, and O. glaberrima* were sent to Cornell University for further evaluation and development. Although all of the donor wild rices belong to the same genome AA as cultivated rice and crossability should not be a problem, however there were some sterility problems and embryo abortion in some cases specially in crosses with *O. barthii* and *O. glaberrima.* Failure of embryo development was observed 10-15 days after pollination. Therefore, embryo rescue was used successfully to overcome this problem, although porcentage of recovery varied among cross combinations. Hybrid plants recovered through embryo rescue were also used in the backcrossing scheme if they had a desirable phenotype.

220 BC2F2 families derived from the cross Oryzica Llanos 5/O. rufipogon were evaluated under field conditions at CIAT-Palmira using a highly viruliferous insect colony of *Tagosodes oryzicola*. M. Distributions of disease incidence (%) based on a 0 - 9 scale (0=no disease symtom; 9=>70% diseased plants) is presented in Fig. 1

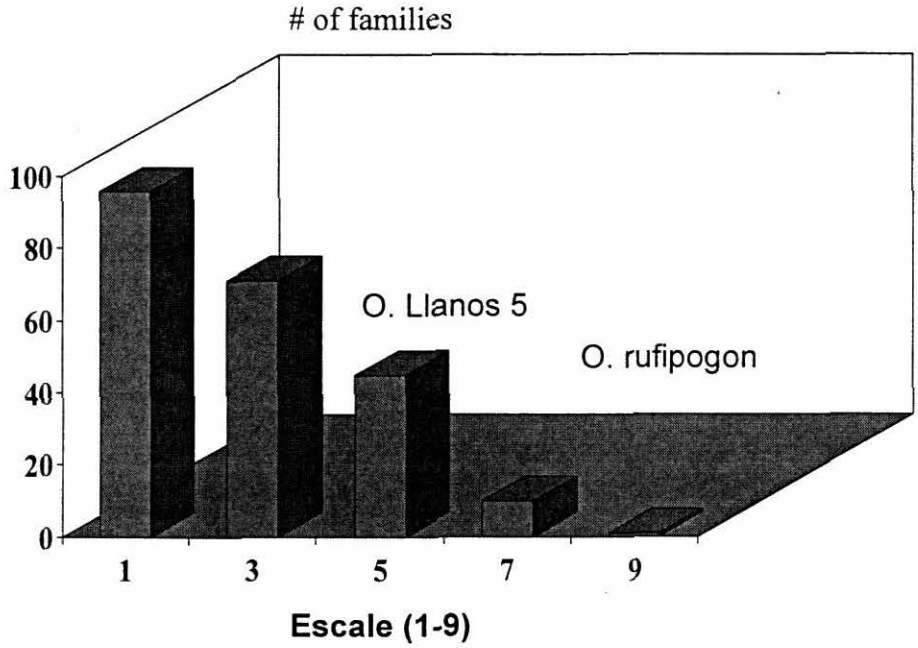


Figure 1. Frequency distribution for rice Hoja Blanca virus disease in 220 BC2F2 Families of the Oryzica Llanos 5/*O. rufipogon cross.*

Transgresive segregation for rice hoja blanca virus resistance can be observed, with approx. 50% of the families falling in the categories 1 and 3. This preliminary data from an un-replicated experiment seem to suggest that positive alleles for rice hoja blanca virus from *O. rufipogon* could be contributing to increased resistance to this particular virus disease. More testing is needed to confirm this result.

Yield Trials and linkage analysis

These evaluations were conducted during June-October 1996 and Dec/96-April/97. We are still processing and recording data taken on main agronomic traits and only partial information generated in CIAT-Palmira is presented. The distribution of grain yield (kg/ha) of 300 BC2F2 families (Bg90-2/*O. rufipogon*) derived from plot yields consisting of 40 plants (20 plants/row x 2row) averaged over two replications is illustrated in Fig. 2.

Transgressive segregation can be observed, with several lines (11%) having between 5 and 25% higher yield than the recurrent parent Bg90-2. Transgressive segregation for other yield components was also observed. Grain yield data taken on 38 BC2F3 families confirmed results obtained in the BC2F2 generation. This preliminary data is in agreement with reports coming from the work being conducted in China at the Hunan Hybrid Rice Research Center (McCouch,

S. 1995) and in South Korea. These data from different groups working with diverse recurrent parents suggest that DNA introgressed from *O. rufipogon* can contribute positively to yields in elite rice cultivars. Evenmore, data from China (Xiao et al, 1996) suggest that two QTLs found in chromosomes 1 and 2 of *O. rufipogon* are responsible for this yield increase.

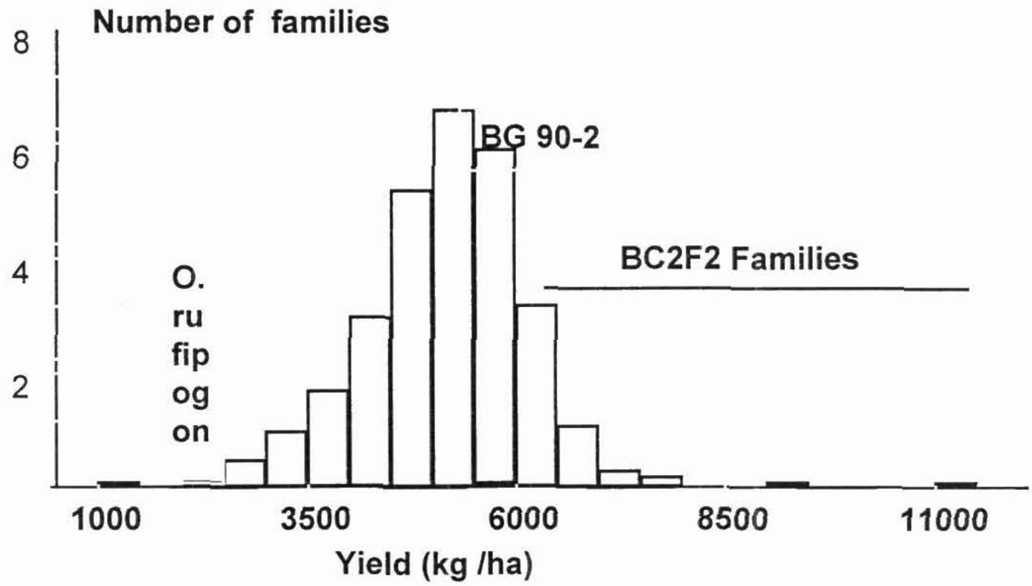


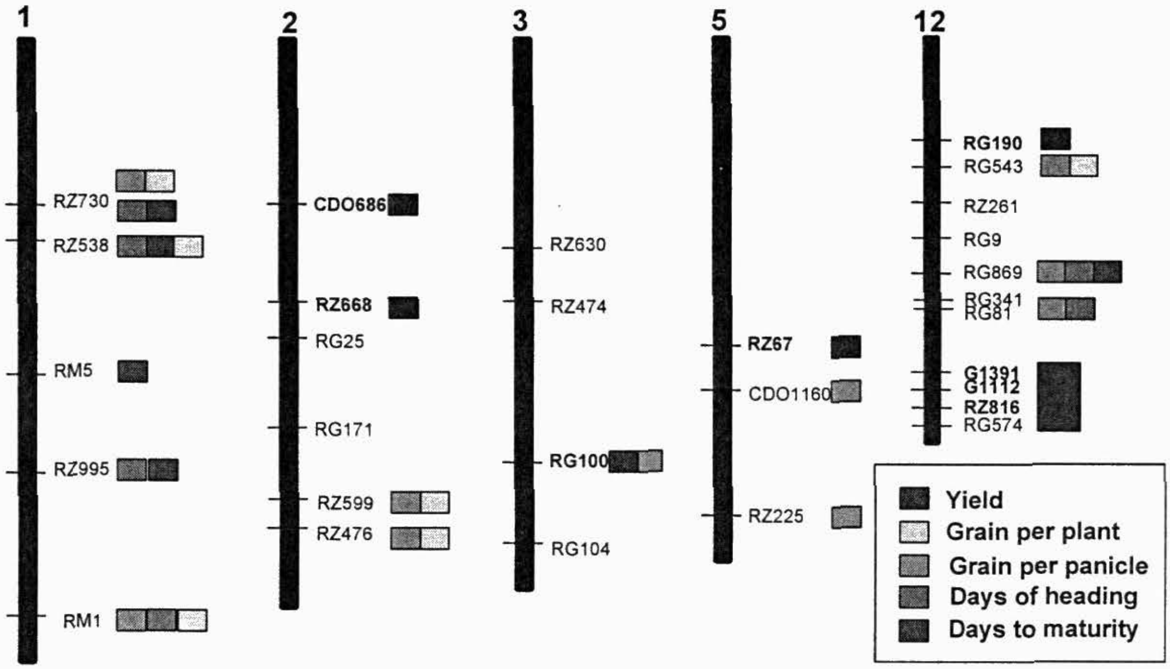
Figure 2. Frequency distribution of grain yield of 300 BC2F2 families from BG90-2/O.

Based on the 52 RFLP and 3 microsatellites from RF-Cornell framework map screened on 303 BC2F2 families putative linkage were identified with yield, yield components from replicated data available for the whole mapping population. Preliminary results using one way anova and t-test indicate associations between markers and yield on chromosome 2 similar to J.Xiao and S. McCouch results (Fig 3). No linkage for yield was detected so far on chromosome 1 as reported by J.Xiao and S. McCouch. Other associations were also identified for yield and the various yield components (Fig 3).

Further work at CIAT will indicate more definitive identification of positive QTLs for yield and whether or not we are dealing with the same QTLs reported by the Chinese group; besides, QTL analysis performed in populations developed with *O. glaberrima* and *O. barthii* will show if they carry the same QTLs for yield found in *O. rufipogon*. If each wild rice posses specific positive alleles for yield, then rice breeders will have a tremendous breeding strategy for increasing yield in a systematic and pyramided manner, that is, in a step-wise process.

In sumary, preliminary data presented support the hypothesis that DNA introgressed from *O. rufipogon* can contribute positively not only to yield in elite rice cultivars but also in terms of stress resistance. This information also provides the basis for implementing the method proposed by Tanksley and Nelson 1996 referred to as "advanced backcross QTL analysis" for the simultaneous discovery and transfer of valuable QTLs from wild germplasm into elite breeding lines.

Fig 3. Preliminary results of positive QTL alleles for yield and yield components



FUTURE ACTIVITIES

1. Complete agronomic and molecular characterization of progenies, and QTL analysis in the Bg90-2/*O. rufipogon* cross to determine number of QTLs associated with yield increase and its expressions across environments using already identified 50 RFLP polymorphic clones and 30 microsatellites.

2. Analysis of other traits and determination of contribution for positive alleles of each of the parents.

3. Development of NiLs to be initiated based on QTL analysis carrying specific QTLs for use in breeding programs.

4. Start agronomic and molecular characterization of several other populations involving crosses with *O. barthii* and *O. glaberrima* to determine the presence of QTLs for yield increase.

5. Training of scientits from national programas in the area of marker-asisted breeding.

6. Estimate the cost of developing improved varieties through the use of molecular markers.

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OUTPUT 1. ENHANCED GENE POOLS

B. RICE IMPROVEMENT: USING GENE POOLS AND POPULATIONS WITH RECESSIVE MALE-STERILE GENE AND CONVENTIONAL BREEDING

Marc Châtel, Yolima Ospina, Jaime Borrero

I. PRESENTATION

1. HIGHLIGHTS

UPLAND SAVANNAS RICE BREEDING

During both semesters of 1996, the activities developed by the upland savannas conventional breeding project were reduced, but reactivated to a certain extent during 1997 A.

Breeding lines were sent to EMBRAPA-CNPAF for observation and seed increase. They were incorporated to their breeding program with which we maintain close collaboration. Visits and exchange of information on the behavior of the CIAT breeding lines are made regularly. Seeds were shipped back to CIAT headquarters and seed increased at Palmira experimental station during the second semester of 1997.

The demand for Upland Rice breeding lines is increasing. New partners were identified (South of Brazil-IRGA, Colombia-Ministry of Agriculture, small farmers of the Atlantic Coast, Argentina-University of Tucuman, Venezuela- FONAIAP and Universidad Nacional Experimental de los Llanos Orientales "Ezequiel Zamora".

UPLAND RICE FOR THE HIGHLANDS OF COLOMBIA

From 1993, we started, with the "Centro Nacional de Investigaciones de Cafe" CENICAFE and the Hillsides Program of CIAT, an informal collaboration on the adaptation of rice as a new crop for the highlands ecosystem of Colombia. Results are very promising (see Upland Rice Improvement for the Highlands of Colombia, 1996 Report).

Two (2) Upland lines were proposed for registration in the rice catalogue of CIRAD. One from savannas Upland rice for medium altitude (1300 masl) , and the other one, introduced from Madagascar (CIRAD/FOFIFA Highlands breeding Project) for high altitude (1600 masl) in the Cauca region

RECURRENT SELECTION BREEDING

CIAT and CIRAD breeding strategies focus on developing and improving populations, phasing-out the development of finish lines. Population development and enhancement aim at providing NARS with potential parents.

The expertise of the collaborative project on recurrent selection is shared with NARS through activity reports, didactic documents, field visits and training courses. The first International course on Rice Recurrent Selection Breeding was held at CIAT in 1996. Fifteen scientists from

13 countries attended the course. Back to their countries, many of them started using recurrent selection in their breeding program during 1997.

Recurrent selection germplasm crosses continents. We shipped populations to Europe (Spain, Hungary and Romania) and Asia (China).

The manual on recurrent selection was translated in English and distributed to non-Spanish speaking countries in Latin America and abroad.

The proceedings of the first International Meeting on Rice Recurrent Selection held in 1995, in Goiania – Brazil, was published by CIAT, EMBRAPA, CIRAD and Fundacion Polar.

A Gene Pool developed by the CIRAD/IRRI Collaborative Rice Project was registered in the germplasm catalogue for recurrent selection as GPIR-22.

THE CIRAD/CIAT/FLAR COLLABORATIVE PROJECT

The third Collaborative Meeting between CIAT, CIRAD, INRA, and ORSTOM was held at CIAT Headquarters in May, 97. The ongoing activities of the CIRAD/CIAT Rice Collaborative Project were confirm and the project was reinforced by:

- The appointment of a new CIRAD scientist at CIAT headquarters, in August, (Dr. Michel Valès.)
- Starting the adaptation of the ADVENTROP software to Latin America in March, (Dr. Thomas Le Bourgeois).

2. BACKGROUND INFORMATION

Genetic uniformity, or lack of genetic diversity, is of major concern to breeders, geneticists, and the agricultural community in general. In many crops, genetic improvement is usually accomplished by reducing genetic diversity in the gene pools used to develop new varieties. But genetic uniformity is now considered as increasing a crop's potential vulnerability to disasters caused by biotic or abiotic constraints. In Latin America, the genetic diversity of rice varieties depends on a small genetic landraces core. A way of broadening the genetic base of Latin American rice and assessing the genotype-by-environment interaction is to identify specific potential parents and pool them to develop new, genetically broad-based, breeding material. CIAT and CIRAD new breeding strategies focus on developing and improving populations to provide sources of potential parents with specific traits required by the national breeding programs. One suitable breeding method to achieve this goal is Recurrent Selection.

Started in 1992, the CIRAD/CIAT rice improvement collaborative project introduced from Brazil and French Guyana, gene pool and populations segregating for a male-sterile recessive gene.

The main objectives of the project are:

(a) To understand the performance of the introduced germplasm in the upland acid soils of the Colombian savannas,

(b) To maintain the germplasm by harvesting fecundated male-sterile plants,

(c) To identify adapted fertile genotypes for use in breeding programs for fixed lines,
(d) To start recurrent selection by recombining the best selected genotypes in the introduced germplasm, and
(e) To create new populations by incorporating the best locally adapted lines of CIAT upland rice breeding program into the best adapted introduced germplasm that provides a good source of male-sterile background.

II. RECURRENT SELECTION FOR SAVANNA and HIGHLANDS UPLAND RICE

Marc CHATEL, Yolima OSPINA and Jaime BORRERO

1. INTRODUCTION

The upland rice recurrent selection project aims at adapting, developing and selecting upland rice gene pools and populations. The main characteristics of the germplasm for Savannas condition are:
- . Tolerance to soil acidity,
- . Resistance to diseases, mainly rice blast (Pyricularia grisea Sacc.),
- . Resistance to pests, mainly Tagosodes orizicolus,
- . Good grain quality (translucent, long-slender grain), and
- . Earliness (total cycle about 115 days)

For the Highlands we are looking for:
- . Tolerance to cold temperatures
- . Grain yield potential
- . Grain quality

2. UPLAND SAVANNAS RICE

The activities reported here were conducted in two different experimental stations:
-Off-season (1996 B): October 1996 to March 1997 at Palmira Experimental Station (EEP)
-Cropping season (1997 A): April to September 1997 at La Libertad Experimental Station (EELL), near Villavicencio (Colombia).
Blast intensity was monitored during 1997 A on the international differential and commercial lines. Blast samples were sent to CIAT Palmira for lineage identification.

2-1. Line Development from Recurrent Populations

During the enhancement of the gene pools and populations using recurrent selection, we selected fertile plants to develop promising fixed lines or potential parents.

2-1-1. Generation S2

The generation S2 comes from fertile S0 plants selected during 1996 A at EELL, and seed increased during 1996 B at EEP.

2-1-1-1. Populations PCT-5\PHB\1\0, PCT-A\PHB\1\0, and PCT-4\PHB\1\1
- Historical
During 1996 A, from the first recurrent selection cycle for leaf blast and Hoja Blanca Virus (see 1996 report 4-2), we selected 211 S0 fertile plants:
49 in PCT-5\PHB\1\0 (11.5% of the total number of fertile plants),
48 in PCT-A\PHB\1\0 (12.4% of the total number of fertile plants) and
114 in PCT-4\PHB\1\1 (17.3% of the total number of fertile plants).
During 1996 B, the S1 generation (211 S1 lines) were grown at EEP and the S2 seeds sent to EELL to grow the S2 generation during 1997 A.

- Cropping Season 1997 A
From the 211 S2 lines evaluated at EELL, 25 were selected.
. PCT-5\PHB\1\0 . 1 line selected (2%)
. PCT-A\PHB\1\0 . 2 lines selected (4%)
. PCT-4\PHB\1\1 22 lines selected (19%)
In each selected line, we selected 6 individual plants.

- Off season 1997 B
The 150 S3 lines (25 families of 6 lines) will be grown at EEP during 1997 B

2-1-2. Generation S4
The generation S4 comes from fertile S0 plants selected during 1995 A, at EELL. The generations S1 and S3 were grown during 1995 B and 1996 B respectively at EEP. The S2 generation was selected during 1996 A at EELL.

2-1-2-1. Populations PCT-5\0\0\0, PCT-A\0\0\0 and PCT-4\0\0\1
- Historical
During 1995 A, cropping season at La Libertad (1995 A, EELL) we selected 55, 85 and 18 S0 fertile plants in PCT-5\0\0\0, PCT-A\0\0\0 and PCT-4\0\0\1 respectively, and during 1995 off-season we grow the S1 at Palmira (1995 B, EEP).

During 1996 A, cropping season a total of 158 S2 and 3 checks (Oryzica Sabana 6, IAC 165 and CIRAD 409 were observed at the EELL and selected. We discarded 102 S2 lines (64,5%) mainly for plant type and yield potential. A total of 56 S2 lines (35.4%) were selected: PCT-5\0\0\0, 21 lines (38.1%); PCT-A\0\0\0, 26 lines (30.6%); and PCT-4\0\0\1, 9 lines (50%). In the 56 selected lines we harvested 178 fertile plants, 62, 91 and 25 in PCT-5\0\0\0, PCT-A\0\0\0 and PCT-4\0\0\1 respectively.

For each selected S2 line, a different selection intensity was applied in relation to the phenotipic value of the lines (grain yield potential, plant and grain type). For example, the highest average selection intensity in three PCT-5\0\0\0 S2 lines was 14% and the lowest average was 1.6% in 14 S2 lines. The S3 generation was grown during 1996 B at EEP and the S4 seeds were sent to EELL to grow the S4 generation during 1997 A.

- Cropping Season 1997 A

From the 178 S4 lines evaluated, 47 were selected

. PCT-5\0\0\0 . 3 lines selected (5%)

. PCT-A\0\0\0 . 35 lines selected (38%)

. PCT-4\0\0\1 . 9 lines selected (36%)

In each selected line, we selected 6 individual plants.

- Off season 1997 B

The 282 S5 lines (47 families of 6 lines) will be grown at EEP during 1997 B.

2-1-2-2. Population PCT-4\0\0\1>S2

- Historical

During 1996 A, we started the enhancement of this population using S2 lines evaluation. We decided to take opportunity of the 1996 A S2 trial to select S2 lines and individual fertile plants for line development. From a total of 152 S2 lines evaluated, we selected 19 (12.5%) and 74 individual plants, based on plant and grain type, and grain yield potential.

During 1996 B, the S3 generation was grown at EEP and the S4 seeds were sent to EELL to grow the S4 generation during 1997 A.

- Cropping Season 1997 A

From the 74 S4 lines evaluated , 16 were selected (22%). In each selected line, we harvested 6 individual plants.

- Off season 1997 B

The 108 S5 lines (16 families of 6 lines) will be grown at EEP during 1997 B.

2-1-2-3. Populations PCT-5\0\0\0, PCT-A\0\0\0 and PCT-4\0\0\1. Plant selection in S3 lines at EEP, 1996 B

-Historical

During 1996 B, at EEP we selected, in S3 lines, 12 individual fertile plant with suitable characteristics. The S4 seed were sown during 1997 A at EELL.

- Cropping Season 1997 A

From the 12 S4 lines evaluated, only 3 were selected in one population.

. PCT-5\0\0\0 .no selection

. PCT-A\0\0\0 .no selection

. PCT-4\0\0\1 .3 lines selected (75%)

In each selected line, we selected 6 individual plants.

- Off season 1997 B

The 18 S5 lines (3 families of 6 lines) will be grown at EEP during 1997 B.

2-1-3. Advanced Generations

The advanced generations come from S0 fertile plants selected in the germplasm we introduced in 1992 from Brazil (with male-sterile gene) and from the gene pool and populations previously developed at CIAT (without male-sterile gene).

2-1-3-1. From populations with male-sterile gene

- Historical

During 1995 B, at EEP we seed increased 2 and 4 advanced lines selected into CNA-IRAT 5 and CNA-IRAT A.

During 1996 A, we observed these 6 lines at EELL. In each of the 6 lines we selected 5 individual plants.

During 1996 B we seed increase the 30 plants at EEP to set-up a yield trial at EELL in 1997 A.

- Cropping season 1997 A

The yield trial was conducted and analyzed. Three lines present a high yield potential and good milling characteristics.

2-1-3-2. From populations without male-sterile gene

- Historical

The first populations for recurrent selection breeding, developed by hand-crossing, were developed by the CIAT Rice Program in the early 90's (Drs. E.P. Guimarães and F. Correa), targeting blast resistance using indica and japonica parents. One Gene Pool and three populations were registered in the recurrent selection catalogue as GPCT-1, PCT-2, and PCT-3. Fixed lines were selected from GPCT-1 and PCT-3 at Santa Rosa experimental station (Hot spot for blast evaluation).

In 1996 A, we selected 89 individual plants showing good characteristics for savannas conditions.

- Cropping season 1997

The 89 progenies were evaluated under Savannas acid-soil conditions at EELL. A total of 36 lines was selected. As these lines comes from indica-japonica recombination, Dr. J. Gibbons from FLAR shows interest for this material.

2-1-4. Upland Line registration

CIAT does not register lines as CIAT lines. When a specific line does well in a country, the national Institution can decide to name and released it for commercial cultivation.

CIRAD has a mechanism by witch breeders can apply for the registration of a specific material in a Catalogue. The line is named CIRAD with its local synonymous in case of collaborative work, as "working material".

- Historical

During 1996, two advanced lines, CNA-IRAT 5 \SA\0\3>127-2-M-2-M and CNA-IRAT A\SA\0\3>1-M-2-M-4-M selected from the populations CNA-IRAT 5 and CNA-IRAT A, were proposed for registration in the rice catalogue of CIRAD. They are registered as CIRAD 410 and CIARD 411, respectively.

- Cropping season 1997

The results of the yield trial show that three lines are very promising. They will be proposed for registration in the CIRAD rice catalogue.

3. POPULATION MAINTENANCE THROUGH RECOMBINATION

3-1. Historical

Until now the maintenance of the upland populations was made under irrigated conditions at Palmira. But, results obtained in Madagascar under theses conditions show that a possible genetic drift towards more indica plant type frequency in the population can occur. Such a drift can be explained by a more effective cross polinization of the genotypes with indica background. We have to remember that the male-sterile line used to buildup the populations is an irrigated indica line (IR 36 mutant).

During the 1996 A cropping season, we decided to maintain and seed increase the upland populations under Savannas conditions. We maintained 6 populations:

CNA-IRAT 5/0/4, CNA-IRAT A/0/2, CNA-IRAT P/1/1, PCT-A\0\0\0, PCT-5\0\0\0, and PCT-4\0\0\1.

All male-sterile plants were identified, harvested individually and their seeds mixed in equal proportion. Fertile plants were also harvested individually and their seeds mixed in equal proportion. The populations were sent to CIAT Palmira and stored in the cold chamber for further use by the program and to attend any request from regional NARS breeding programs.

3-2. Cropping season 1997

As we have enough seed, no new maintenance of this germplasm was made unless for PCT-5 population.

4. POPULATION ENHANCEMENT - RECURRENT SELECTION

CIAT's rice project emphasizes the enhancement of populations and is slowing down the production of fixed lines for direct release by the NARS of the region. The strategy is to develop and enhance gene pools and populations for well-targeted trait(s) to be used as source of potential parents by the NARS ' breeding programs. In the first two years of the recurrent selection project started in 1991, we focused on the introduction of germplasm from Brazil (CNPAF and CIRAD) and French Guiana, its characterization and mass selection. From 1995, we concentrated our activity on the enhancement and development of new populations.

4-1. Recurrent Selection based on S2 line evaluation: Population PCT-4\0\0\1

4-1-1. Historical

During 1995 A, at EELL, 159 S0 fertile plants were selected.

During 1995 B, the generation S1 was grown at EEP.

During 1996 A, we started the first recurrent selection cycle.

Evaluation: 152 lines S2 and 2 checks (Oryzica Sabana 6 and CIRAD 409 were evaluated and selected at EELL under the "Augmented Blocks" statistical design.

Selection: Results from the S2 trial were analyzed and 53 S2 were selected.

Recombination: Remnant seeds of the S0 plants that originated the selected S2 lines were mixed and grown to develop the recombined enhanced population. The enhanced recombined population was identified as PCT-4\SA\1\1.

4-1-2. Cropping season 1997 A

Population PCT-4\SA\1\1 was grown at EELL to go through a second selection cycle.

4-1-2-1. Selection of fertile plants

A total of 155 S0 plants were selected. A sample of each S0 seed was kept-up in the cold chamber. The S1 generation will be grown during 1997 B, at EEP, and S2 seeds harvested. The S2 lines are to be evaluated during 1998 A, at EELL.

4-1-2-2. Harvest of the male-sterile plants

The male-sterile plants were harvested individually and their seeds mixed in equal proportion to complete the second cycle of recombination of the population selected one time. The identification of the second cycle of recombination is identified as : PCT-4\SA\2\1. The seeds will be kept in cold chamber for future use.

4-2. Mass Recurrent selection on both sexes for main agronomic traits, blast and Hoja Blanca: Populations PCT-4\0\0\1, PCT-A\0\0\0 and PCT-5\0\0\0.

4-2-1. Historical

During 1995 A, at EELL, we eliminated all the plants showing symptoms of leaf blast and HBV at the vegetative stage. At harvest time we selected male-fertile plants having good phenotype. Seeds produced by these plants are the result of the fertilization by the pollen produced by heathy fertile plants. 102, 99 and 96 male-sterile plants were selected into PCT-5\0\0\0, PCT-A\0\0\0 and PCT-4\0\0\1 respectively and their seeds mixed in equal proportion. The identification of the first mass recurrent selection cycle (selection and recombination) was: PCT-5\PHB\1\0 - PCT-A\PHB\1\0 and PCT-4\PHB\1\1

During 1996 A, the seed mixture of each population with one mass recurrent selection cycle was grown at EELL. For the second recurrent selection cycle the same selection method as during 1995A was applied. 304, 341 and 442 healthy male sterile plants fertilized by the pollen of fertile healthy plants were selected into PCT-5\PHB\1\0, PCT-A\PHB\1\0 and PCT-4\PHB\1\1 respectively and their seeds mixed in equal proportion. The identification of the second mass recurrent selection cycle (selection and recombination) is: PCT-5\PHB\1\0,PHB\1 - PCT-A\PHB\1\0,PHB\1 and PCT-4\PHB\1\1,PHB\1

4-2-2 Cropping season 1997

During 1997 A, the seed mixture of each population with two mass recurrent selection cycle was grown at EELL. For the third recurrent selection cycle, the same selection method as during 1995 A and 1996 A was applied (elimination during the vegetative stage of all plants with symptoms of leaf blast and Hoja Blanca). 218, 253 , and 165 healthy male sterile plants fertilized by the pollen of fertile healthy plants were selected into PCT-5\PHB\1\0,PHB\1 - PCT-A\PHB\1\0,PHB\1 and PCT-4\PHB\1\1,PHB\1 respectively and their seeds mixed in equal proportion. The identification of the third mass recurrent selection cycle (selection and recombination) is:

PCT-5\PHB\1\0,PHB\1,PHB\1, PCTA\PHB\1\0,PHB\1,PHB\1 and PCT-4\PHB\1\1,PHB\1,PHB\1.

5. DEVELOPMENT OF NEW POPULATIONS

The development of new populations is one of the basic activities of the project. It is the main source of new recombined variability for population enhancement and line development. We have to be well focus on the choice of the variability we will gather and recombine in a new germplasm as well as what source of male-sterility to be used, (generally it comes from a well-adapted existing population or gene pool).

In 1996 B, we decided to buildup at EEP, two (2) new *japonica* populations targeting upland savannas and hillsides ecosystems. The source of male-sterility background is the best *japonica* population developed earlier by the project.

5-1. Upland Savannas population

The idea in developing that population is to pool the best lines of the CIAT conventional rice breeding project and the commercial varieties released in Brazil, Colombia and Bolivia.

5-1-1. Historical

In 1996 B, eighteen (18) lines were selected based on their behavior for earliness, blast and acid soil tolerance, and grain quality. Male-sterile plants from the best-adapted upland japonica population (PCT-4) were used as female parent. Each line was crossed with at least 4 male sterile-plants of the population PCT-4.

5-1-2. Cropping season 1997

During 1997 A, at EEP, each resulting F1 was grown individually, evaluated, and individual plants selected. The F2 seeds of the selected F1 plants were bulked in equal proportion. Each F2 bulk was mixed in balanced proportion to buildup the new basic population identified as PCT-11\0\0\0.

During 1997 B, at EEP, the basic population will be recombined one time . The identification of the first cycle of recombination of the basic population will be : PCT-11\0\0\1.

5-2. Upland hillside population

The idea is to develop a population for the Andean highlands of Colombia, with earliness and cold tolerance for high altitude, 1300-1600 masl.

5-2-1. Historical

In 1996 B, eleven lines - 6 from the CIRAD/FOFIFA hillsides program of Madagascar, 4 from the CIAT upland savannas program and 1 IRAT line - were selected based on their previous evaluation at high altitude. We used the best-adapted upland japonica population (PCT-4) as source for male-sterility. Each line was crossed with at least 4 male sterile-plants of PCT-4.

5-2-2. Cropping season 1997

During 1997 A, at EEP, each resulting F1 generation was grown individually, evaluated, and individual plants selected. The F2 seeds of the selected F1 plants were bulked in equal proportion. Each F2 bulk was mixed in balanced proportion to buildup the new basic population identified as PCT-13\0\0\0.

During 1997 B, at EEP, the basic population will be recombined one time. The identification of the first cycle of recombination of the basis population will be: PCT-13\0\0\1.

6. REGISTRATION OF NEW POPULATIONS

In 1997, one (1) Gene Pool developed by hand-crossing (without using male sterility) for durable resistance to blast was proposed for registration in the Recurrent Selection Catalogue. It was buildup in the Philippines by Dr. Brigitte Courtois, in the framework of the collaborative project between CIARD and IRRI. It was registered as GPIR-22.

7. UPLAND RECURRENT SELECTION GERMPLASM DISTRIBUTION

From 1995 we started to release recurrent populations and gene pools to Latin American NARS. The Countries where the germplasm was shipped are: Bolivia, Brazil, Colombia Cuba and Venezuela.

III. CONVENTIONAL BREEDING FOR SAVANNA and HIGHLANDS UPLAND RICE

1. SAVANNA UPLAND RICE

Marc CHATEL, Yolima OSPINA and Jaime BORRERO

As stated earlier, the objective is to phase-out gradually the development of fixed lines for direct release by NARS. But we are keeping some activities. **In 1996 B**, we sent 537 savannas upland lines (F4 and F5 generation) to EMBRAPA Arroz e Feijão, for observation and seed increase. These lines were sent back to CIAT Palmira **in 1997.**

1-1. Upland lines from Brazil

We received from EMBRAPA Arroz e Feijão a set of Brazilian lines for evaluation under acid-soil condition.

1-2. Use of CIAT/CIRAD Savannas Lines in Brazil

During our visit to EMBRAPA Arroz e Feijão, we had the opportunity to track the use of CIAT lines in the breeding program of the Center. It is clear that CIAT/CIRAD Savannas material is very useful for the Brazilians, at each step of their breeding project. In average, 57% and 57.8% of the lines present in the trials and the segregating generations, respectively, comes from CIAT/CIRAD (table 1). A special mention has to be made witch relates to the use of CIAT/CIRAD lines as parents. Fifty one percent (51.1%) of the parents used by EMBRAPA Arroz e Feijão are CIAT/CIRAD lines. The main characteristics they like from our lines are, grain quality and plant type.

1-3. New Partners interested in Savannas Upland Lines

During 1997 B, we seed increased the lines we received back from Brazil as well as from CIRAD, to be able to attend the demand from new partners interested in Latin American breeding or fixed lines. The new partners are; Colombia (Atlantic Coast), Brazil (South, IRGA), Argentina (Region of Tucuman), and China (Yunnan and Jiangxi Provinces).

A special statement has to be made for China. In 1995, we sent the first set of Savannas lines from Brazil and CIAT/CIRAD to this country in the framework of a collaborative project between The Foods Crops Research Institute (Yunnan Academy of Agricultural Science, KUNMING) and CIRAD. The first results are very promising showing good adaptation and acceptability. This year, Dr, Tao Dayun sent us a letter telling us that a CIAT/CIRAD Savannas Upland Line (CT 9278-11-14-2-1-M) is a strong candidate for direct release in his country during 1998.

1-4. Seed multiplication

During 1996 and 1997, the demand for Savanna Upland seed in the "Llanos Orientales" of Colombia was very high. Not enough seed was available in the market. CORPOICA decided to seed increase the two released lines: Oryzica Sabana 6 and 10. From our part we started G0 generation at EELL.

1-5. Line release

"Linea 30", identified as a very promising line since 1995, was never released because of the adjustment plan of CORPOICA Regional 8. The line was registered as CIRAD 409, last year. CORPOICA is willing to release the line for the Meta State in 1998. In 1997 we started the production of genetic seed.

2. HIGHLANDS UPLAND RICE

Marc CHATEL, Argemiro MORENO-B, Jaime BORRERO and Carlos QUIRÓS

The Andean mountain range runs across Colombia from south to north raising as high as almost 6,000 masl. The most important agricultural activity in the mid altitudes (1,000-2,000 masl) across this area is coffee, mainly planted by small farmers. This crop requires at least three years to start commercial production. In the meantime farmers use considerable resources in weed control and to prevent erosion. With this crop system in mind, CENICAFE has been working in different crop diversification alternatives to help farmers to have incomes before coffee starts production. It is also worth mentioning the agricultural research developed with small farmers in the Cauca State – South West of Colombia – where new crops have to be incorporated in the existing or new cropping systems developed by CIAT to ensure local food security and where new upland rice lines with adaptation to the climatic conditions of midaltitude farms look promising..

2-1 Historical

With the objective of identifying upland germplasm adapted to the hillsides areas of Colombia, the CIAT/CIRAD rice project together with CENICAFE, initiated in 1993 the evaluation of 31 selected savannas lines in the heart of the coffee growing area, at 1,300 masl. The climatic data collected at the main site show average temperatures ranging from 23.1 to 20.6 C. The monthly average maximum (28.5 C) occurs in February and the minimum (16.9 C) in September. Therefore, the germplasm for this region has to show cold tolerance and low spikelet sterility. The lines to compose this initial trial were selected in the Savannas Upland germplasm of CIAT, based on previous knowledge gained from the CIRAD/FOFIFA Highland Rice Project in Madagascar. Upland lines have to be early and cold tolerant (measured by the fertility of the panicle). Results obtained in La Catalina, State of Risaralda, show that the percentage of empty grains ranged from almost 100% to 12%, indicating that the germplasm presents variability for cold tolerance. Growth duration was extends to around 150 days after sowing (DAS) compared to 120 DAS under Savannas conditions. The selection concentrated in the lines with at least 60% fertility. The average grain yield of the six (6) best-adapted lines was higher than expected, ranging from 3,775 to 5,592 hg/ha.

In 1993, upland lines developed by CIRAD/FOFIFA for the highlands of Madagascar were introduced to Colombia and seed increased. The new germplasm was distributed to CENICAFE and the Hillsides program of CIAT. ***In 1994***, line evaluation started in the Cauca State. ***In 1995***, the Centro Internacional de Agricultura Organica (CIAO) started the evaluation at 1,600 masl. The first results were presented at the Conference on Rice for the Highlands in Madagascar in April 1996.

2-2. Cropping seasons 1996-1997

2-2-1. Coffee Region – CENICAFE and CIAO

The line CT 10069-27-3-1-4, well adapted to medium-altitude was used in a trial with young coffee trees. Considering the potential of these line through the different years (average grain yield of 4Tons/ha), we decided to apply for registration in the CIRAD Rice Catalogue.

2-2-1-1. Germplasm introduction

Forty-one (41) new lines were introduced from Madagascar to Colombia and seed increased at CIAT-Palmira, and then dispatched to CENICAFE and CIAO. The same set of 41 introduced lines was dispatched to the CIAT Hillsides project for testing in Colombia and Central America.

2-2-1-2. Crosses

Eleven single crosses were made at EEP, between line CT 10069-27-3-1-4 and 10 lines from Madagascar and CIRAD, previously selected for their good behavior at highlands condition. The F1 generation was grown during 1997 A at EEP.

2-2-2. Cauca State and Central America

In the Cauca State, the five (5) best lines selected last year and one Savannas upland check (CIRAD 409), were tested at on farm level by five smallholders. The results are presented in the table 2. The best line from last year experiment (Latsidahy/FOFIFA 62-3) is also the best one at on farm level, with an average grain production of 1,400 kg/ha at "La Laguna " site at 1600masl. The Savannas upland check shows complete sterility. If the line confirms its results this semester, we will apply for registration in the CIRAD Rice Catalogue.

A survey was conducted with the five farmers to know what are the most desirable characteristics of a rice line. The results are presented in the table 3. Farmers are ranking, at the first and second place, the high number of panicle and short cycle as the best characteristics.

IV. RECURRENT SELECTION FOR LOWLAND RICE

1- INTRODUCTION

The Recurrent Selection breeding project started in 1991 with the introduction in Colombia of different gene pools and populations developed in Brazil by EMBRAPA Arroz e Feijão and by CIRAD, and in French Guiana by CIRAD. The germplasm was characterized at CIAT Palmira and the best-adapted populations were used to develop new populations by the incorporating new variability. This resulted in three (3) populations that were registered in the recurrent selection catalogue as PCT-6, PCT-7 and PCT-8. This work was conducted at CIAT in close collaboration with Drs. C. Martínez and E.P. Guimarães. A gene pool was also built-up using a different gene of male sterility. The gene pool was registered as GPCT-9. Finally a second gene pool from CIRAD was registered as GPIRAT-10.

2- FLAR-CIRAD RECURRENT SELECTION FOR HOJA BLANCA VIRUS

Marc CHATEL, James GIBBONS, Jaime BORRERO and Monica TRIANA

2-1. Introduction

One of the objectives of FLAR is to focus on breeding. Recurrent selection is an alternative method to traditional breeding and is being incorporated in the FLAR breeding activities.

We decided to apply the recurrent selection breeding method to the existing germplasm for Hoja Blanca Virus and Blast resistance.

2-2. Cropping season 1997

Three populations, PCT-6\0\0\2, PCT-7\0\0\0, and PCT-8\0\0\0 and the gene pool GPCT-9\0\0\0F were evaluated for Hoja Blanca virus according to the methodology developed at CIAT.

Each germplasm and checks were sown in the "Hoja Blanca nursery". Fourty five (45) days after sowing, the populations and the checks were evaluated, counting the number of healthy and diseased plants. The results are presented in the table 4. In the nursery, the four original germplasm show medium susceptibility to Hoja Blanca (same level as the check Oryzica 1). After transplanting, PCT-7 and GPCT-9 presented a high number of plants with Hoja Blanca symptoms. The two less susceptible germplasm are PCT-7 and PCT-8, with 18 and 19% of immune plants, respectively. These plants will be recombined, completing the first cycle of recombination. The healthy plants of each germplasm were transplanted separately for recombination on male-sterile plants.

The selected populations are identified as:

PCT-6\HB\0\2, PCT-7\HB\0\0, PCT-8\HB\0\0 and GPCT-9\HB\0\0F.

The recombined populations, after the first cycle of selection, are identified as: PCT-6\HB\1\2, PCT-7\HB\1\0, PCT-8\HB\1\0 and GPCT-9\HB\1\0F.

Each germplasm with one cycle of selection-recombination will be evaluated for blast resistance at Santa Rosa Experimental Station (Hot spot for blast) during 1998.

3. RECURRENT SELECTION IN COLOMBIA

Hernando DELGADO, Marc CHATEL and Yolima OSPINA

Last year we sent four sets of germplasm (populations PCT-6\0\0\2, PCT-7\0\0\0, and PCT-8\0\0\0 and the gene pool GPCT-9\0\0\0F) to CORPOICA Regional 8 -State of Meta. Each set was grown separately at the "Estación Experimental La Libertad" for recombination, characterization and selection of fertile plants for line development. The four sets of germplasm behave well. The populations PCT-6 and PCT-7 presented the best potential for the future. Next year the best-adapted germplasm will be evaluated for blast resistance at Santa Rosa Experimental Station.

4. RECURRENT SELECTION IN COSTA RICA

Randolph CAMPOS, Marc CHATEL and Jaime BORRERO

In 1996, we sent to Costa Rica the *indica* Gene Pool GPCT-9 and the Population PCT-7. The same year, Randolph Campos of the "Programa Nacional de Arroz" attended the International course on Recurrent Selection held at CIAT. The germplasm was characterized under the Costa Rican condition and was maintained by harvesting male-sterile and fertile plants, independently. In 1997, the germplasm was used for line development by selecting S0 fertile plants.

5. RECURRENT SELECTION IN EL SALVADOR

Ramon Eduardo SERVELLON, Marc CHATEL and Jaime BORRERO

In 1995 we sent three populations (PCT-6, PCT-7 and PCT-8) and the gene pool GPCT-9 to the "Centro Nacional de Tecnología Agropecuaria y Florestal" (CENTA).

- Line development: In 1996, 141 and 97 S0 fertile plants were selected in PCT-7 and PCT 8 respectively.
- Population enhancement for grain yield, plant type, blast, and grain quality: The recurrent selection method used is based on S2 progenies evaluation and recombination from the remnant S0 seeds. One hundred (100) S2 lines from the population PCT-7 are tested in two sites.
- New population development: A population named CNA IRAT ES 1/0/2 was developed by introduction of 4 lines (X-10, CENTA A-1, CENTA A-2 and CENTA A-5) into the Brazilian population CNA IRAT 4/0/6. The new population passes through 2 cycles of recombination and S0 fertile plants were selected during the second semester of 1997.

6. RECURRENT SELECTION IN PANAMA

Ariel E. JAÉN SANCHEZ, Marc CHATEL and Jaime Borrero

In 1996, we sent to Panama the indica Gene Pool GPCT-9 and the Population PCT-7. The same year, Ariel E. Jaén Sánchez, of the "Universidad de Panama, Facultad de Ciencias Agropecuarias", attended the International course on Recurrent Selection held at CIAT. The introduced germplasm was grown and its characterization started. But, due to water shortage, it was not possible to complete the work. Nevertheless, in each material, the earliest S0 fertile plants were harvested. A new sample from the recurrent populations was sent to Panama.

7. RECURRENT SELECTION IN VENEZUELA

Eduardo GRATEROL, Marc CHATEL and Yolima OSPINA

After attending the International Rice Recurrent Selection Course, three Populations (PCT-6, PCT-7 and PCT-8) and three Gene Pools (IRAT 1/420P, IRAT MANA and GPCT-9) were sent to Eduardo Graterol, for characterization under local condition in Calabozo, Guárico State. The objective is to select the best-adapted germplasm to start a recurrent selection program. The traits evaluated in each germplasm were: Flowering time, Tillering ability, Plant height and Disease tolerance (Leaf and Neck blast, Brown spot, Sheath blight and Sheath rot). Two Populations, PCT-6 and PCT-7, were selected and used as source of male-sterile background to develop two new local Populations named as PFD-1 and PFD-2.

8. RECURRENT SELECTION IN THE SOUTHERN CONE

8-1.Recurrent selection in Argentina

Maria Antonia MARASSI, Juan Eduardo MARASSI, Marc CHATEL and Jaime BORRERO

In December 1996, we supplied the "Universidad de Corrientes" with the populations PCT-6\0\0\0,PCT-7\0\0\0,and PCT-8\0\0\0. They were sown at the experimental field of the Company "La Arrocera Argentina" in Villaguay State of Entre Rios. They were observed and characterized.

The maintenance of the populations was made by harvesting the male-sterile plants. The resulting populations are identified as PCT-6\0\0\1, PCT-7\0\0\1, and PCT-8\0\0\1. S0 fertile plants showing good potential were selected and harvested individually for linr development. Seventeen fertile plants, (17) fourteen (14), and thirty-four (34) plants were selected, respectively.

They received the following identification:
PCT-6>Arg-1 to 17, PCT-7>Arg-1 to 14, and PCT-8>Arg-1 to 34.

During the second semester of 1997 it is planed to develop local populations by crossing 6 varieties (IRGA 417, CYPRESS, R.P.2, TAIM, Don Juan INTA and CH4-7) with male-sterile plants of each introduced population.

Argentina has a project for the development of the "Pampa" region, where climatic conditions are similar to those of Chile (same Latitude as Chillan City, 400 km South of Santiago). In this contest, the "Universidad de La Plata" is very interested in the activities we have with the Chileans. The gene pool GPIRAT-10, and the population PQUI-1, as well as the Chilean and European lines can be useful for this region.

8-2. Recurrent selection in Chile

Roberto ALVARADO, Santiago HERNAIZ, Marc CHATEL and Jaime BORRERO

In 1996, we sent to Chile the *japonica* Gene Pool GPIRAT-10 specially developed by CIRAD for temperate climate. The same year, Santiago Hernaiz from INIA-Quilamapu attended the International course on Recurrent Selection held at CIAT. In 1997 the gene pool was grown for characterization and selection of the best fertile plants for line development. It was also used as source of male sterility to build-up a local population by crossing five selected Chilean lines (Qui. 67108, Diamante, Buli, CINIA 609 and CINIA 606), with male-sterile plants of the gene pool. Part of the hybrid seeds was sent to CIAT Palmira for growing the F1 generation (in Chile there is only once cropping season a year). The F2 seeds will be shipped back to Chile in September 1997. The basic Chilean population was named as PQUI-1\0\0\0. During the second semester, 1997 B, at CIAT Palmira we will go through the first cycle of recombination to ensure seed increase for future use. The first cycle of recombination will be identified as PQUI-1\0\0\1.

8-3. Recurrent selection in Uruguay

Fernando PÉREZ DE VIDA, Marc CHATEL and Jaime BORRERO

In 1996, we sent to Chile the *japonica* Gene Pool GPIRAT-10. The same year, Fernando PÉREZ DE VIDA from INIA-Treinta y Trés attended the International course on Recurrent Selection held at CIAT. In 1997 the gene pool was grown for characterization and selection of the best fertile plants for line development. It was also used as source of male sterility to build-up a local population by crossing selected Uruguayan lines with male-sterile plants of GPIRAT-10.

9. RECURRENT SELECTION GERMPLASM MAINTENANCE

As we are managing the catalogue for registration of the Rice Recurrent Selection germplasm, we have the responsibility to have enough seed of the populations. The following germplasm was seed increased during 1997 A, at EEP:

IRAT MANA, IRAT LULU, GPIRAT-10, GPCT-9, PCT-8, PCT-7 and PCT-6.

10. LINE DEVELOPMENT THROUGH ANTHER CULTURE

Zaida LENTINI, Marc CHATEL and James GIBBONS

In 1994, we introduced from French Guyana, the population IRAT-CT. This population comes from the enhancement of the *indica* gene pool GPCNA-18 for anther culture response.

10-1. Historical

One cycle of selection-recombination, for anther culture response, was previously made in Brazil at EMBRAPA Arroz e Feijão. This originated the population identified as IRAT-CT.
From 1995, the CIAT anther culture laboratory processed the population IRAT-CT and ////// R2 lines were developed.

10-2. Cropping season 1997

The //// R2 lines were evaluated by FLAR at Santa Rosa Experimental Station and //// lines were selected.

ACKNOWLEDGMENTS

This document reports the research activities developed during the second semester of 1996 and the first semester of 1997, at CIAT headquarters (Palmira, State of Valle-Colombia), and La Libertad, (Villavicencio, State of Meta-Colombia), experimental stations.

In Colombia, we maintain close collaboration with CORPOICA and CENICAFE.

At regional level in Latin America, research activities were conducted in close collaboration with scientists of different Institutions and Universities.

We would like to acknowledge for their excellent work and collaboration the following persons:

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Emilio da Maia de Castro	Brazil, EMBRAPA Arroz e Feijão
Elcio Perpetuo Guimarães	Brazil, EMBRAPA Arroz e Feijão
Roberto Alvarado	Chile, INIA Quilamapu
Santiago Ignacio Hernaiz Lagos	Chile, INIA Quilamapu
Hernando Delgado Huertas	Colombia, CORPOICA Regional 8
Randolph Campos Morera	Costa Rica, Ministerio de Agricultura y Ganadería
Ramón Eduardo Servellón	El Salvador, CENTA
Ariel E. Jaén Sánchez	Panamá, Universidad de Panamá
Alberto Herrera G.	Venezuela, Universidad UNILLEZ
Eduardo Graterol	Venezuela, DANAC – Fundación Polar
Fernando B. Pérez de Vida	Uruguay, INIA Treinta y Trés

Table 1. Use of CIAT/CIRAD Savannas upland lines in BRAZIL

Yield trials

Trial	Total # of lines	Checks #	CIAT/CIRAD lines	CIAT/CIRAD Participation(%)
Observation	159	4 (1 CIAT)	27	18.0
Preliminary II	36	3	25	75.7
Preliminary III	49	2 (1 CIAT)	39	85.1
Advanced II	25	5 (2 CIAT)	12	60.0
Advanced III	20	5	7	46.6

Segregating generations

Generation	# of lines	CIAT/CIRAD lines	CIAT/CIRAD Parents	CIAT/CIRAD Participation (%)
F6	891	21	-	2.3
F4	1179	672	-	57.8
F3	555	-	-	-
F2	104	-	40	38.5
F1	141	-	72	51.1

Table 2. Highlands Upland Rice, on farm trial. "La Laguna ", 1600 masl, Cauca State. (results from C. Quiroz CIAT)

Lines	Grain yield (Kg/Ha) Farm #1	Grain yield (Kg/Ha) Farm #2	Grain yield (Kg/Ha) Farm #3	Grain yield (Kg/Ha) Farm #4	Grain yield (Kg/Ha) Farm #5	Average grain yield (Kg/ha)
LATSIDAHY / FOFIFA 62 –2	900	1600	650	775	*****	981.25
FOFIFA 116 / SHIN EI	1050	1425	500	*****	-	991.67
LATSIDAHY / FOFIFA 62 –1	262	1785	500	550	*****	775.00
LATSIDAHY / FOFIFA 62 –3	1250	3200	550	1300	720	1404.00
FOFIFA 116 / SHIN EI – 3	1025	-	*****	800	*****	912.50
CIRAD 409	*****	*****	*****	*****	-	*****

***** Complete Sterility
- Not tested

Table 3. Acceptability of upland rice lines. Survey conducted at farm level, "la Laguna, Cauca State. (results from C. Quiroz, CIAT)

Favorable and unfavorable characteristics according farmers comments					
FAVORABLE			UNFAVORABLE		
Characteristic	Frequency	Ranking	Characteristic	Frequency	Ranking
High # of panicle	46.2%	1	Few panicle	38.1%	1
Short cycle	23.1%	2	Long cycle	28.6%	2
Plant type	15.4%	3	Heterogeneity	14.3%	3
Homogeneity	7.7%	4	Small plants	4.8%	4
Heavy panicle	3.8%	5	Small panicle	4.8%	4

Acceptability by farmers of the 6 upland rice lines at vegetative stage

Line Identification	Score	Ranking
LATSIDAHY / FOFIFA 62 –2	17	3
FOFIFA 116 /SHIN EI	15	5
LATSIDAHY / FOFIFA 62 –1	21	2
LATSIDAHY / FOFIFA 62 –3	23	1
FOFIFA 116 /SHIN EI – 3	7	6
CIRAD 409	17	3

Table 4. Recurrent Selection on booth sexes for Hoja Blanca: Germplasm evaluation and plant selection for recombination.

Recurrent Selection Germplasm

Germplasm Identification	# and % of plants showing Hoja Blanca symptoms in the nursery	# and % of plants showing Hoja Blanca symptoms after transplanting	# and % of immune transplanted plants for recombination
PCT-6\0\0\2	2800 (75%)	517 (14%)	416 (11%)
PCT-7\0\0\0	2670 (62%)	850 (20%)	762 (18%)
PCT-8\0\0\0	2423 (72%)	298 (9%)	629 (19%)
GPCT-9\0\0\0F	2727 (66%)	1096 (26%)	331 (8%)

Checks

Line Identification	Plants showing Hoja Blanca symptoms (%)	Immune plants (%) And reaction type	
CICA 8	99.70	0.30	S
COLOMBIA 1	7.48	92.51	R
BLUBONET 50	99.58	0.42	S
ORYZICA 1	68.49	31.50	MS
METICA 1	88.21	11.79	S
CARIBE 8	95.77	4 .23	S
ORYZICA LLANOS 5	16.71	83.29	R

Output 1. ENHANCED GENE POOLS

C. THE USE OF ANTHER CULTURE AND EMBRYO RESCUE FOR ENHANCEMENT OF GENE POOLS

1. Use of embryo rescue to increase recovery of hybrid plants from rice X wild species crosses

- Total of 174 embryos from 7 crosses were processed by embryo rescue.
- Recovery response varied from 23% to 100% depending on the cross.

Embryo rescue was used to aid the recovery of plants from interspecific hybrids, which showed high degree of sterility. A total of 174 individual embryos from 7 different crosses were cultured 3-5 days after pollination when the embryos were between 1-2 mm in size. Smaller embryos did not survive the culture process, and larger embryos aborted . The response varied from 23% to 100% depending on the cross. No difference in response was noted with reciprocal crosses. Collaborators: Adriana Mora (IP4), César Martínez (IP4, SB2), Zaida Lentini (IP4, SB2)

Table 1.- Rice interspecific hybrids recovered by embryo rescue.

Cross	Embryo cultured	Plants produced	Recovery (%)
Jefferson X *O. rufipogon*	53	26	49
Jefferson X *O. bartii*	39	21	54
O. bartii X Jefferson	4	2	50
Jefferson X *O. glaberrima*	18	10	55
Lemont X *O. bartii*	40	14	35
O. bartii X Lemont	17	4	23
Cypress X *O. bartii*	3	3	100

2. Use of anther culture to fix enhanced traits in backcrossed populations from rice X wild species hybrids.

- Twenty one crosses of *O. bartii* /4*Oryzica 3 were processed through anther culture from the first date of sowing.
- The crosses were represented by 117 plants selected in the field from a total of 527 plants.
- Callus induction of these materials varied from 0% to 100% depending on the cross. Plant regeneration of the callus is still in progress.
- Materials from the second date of sowing will be cultured by the end of 1997.

We had used anther culture (AC) as a mean to accelerate the introgression of genes into germplasm adapted to the various rice ecosystems in Latin America. This is the first time we used AC to facilitate the fixing of desirable traits from interspecific hybrids. The crosses cultured were the fourth backcross to the *O. sativa* parent, generated by using as the mother plant different individual plant selections from the third backcross. Oryzica 3 (*O.sativa*) does not respond to AC , and *O. bartii* showed a low callus induction (9%) but high level of green plant regeneration (55.8% $\pm$ 7.6). Doubled haploid lines produced will be evaluated in the field for agronomic traits, and analyzed by molecular markers to identified potential markers associated with the transgressive segregation seen on yield on some of the parental crosses in the third backcross.

Collaborators: Adriana Mora (IP-4); César Martínez (IP-4, SB-2); Zaida Lentini (IP-4, SB-2).

3. Use of anther culture to develop a population for molecular gene tagging of cold tolerance for FLAR members.

- This is a project still pending since the crosses were not produced by FLAR partners this year.
- Instead, twenty three advanced lines from Brazil were cultured with the aim of fixing a promising line which is still segregating for plant type.
- Total of 2138 plants were produced, with an efficiency of 1.7 plants per 100 cultured anthers.

Initially this activity was programmed to produced doubled haploid lines from crosses designed for tagging cold tolerance gene(s) by molecular markers. However, these crosses were not produced by FLAR partners this year. Instead, it was decided to help IRGA rice program in generating doubled haploids from the advanced promising line IRGA 959 at F8 generation, which surprisingly is still segregating for plant type. Self seed from one panicle of each of 23 selected plants were planted in a row in the field. Panicles within each row were harvested and cultured separately in the laboratory. A total of 127,750 anthers were cultured from the 23 selected plants generating a total of 2138, yielding as efficiency of 1.7 plants per 100 cultured anthers, typical of *indica* type of rice. Doubled haploids plants will be evaluated in the field in Colombia, and R3 seeds will be send to Brazil for the final selection of the fix line to be released as a variety for Rio Grande do Sul.

Collaborators: Daniel González (FLAR); James Gibbons (FLAR); Adriana Mora (IP-4); Zaida Lentini (SB-2, IP-4); Oneides Ovazoni. Instituto Rio Grandense do Arroz (IRGA). Porto Alegre, Brazil.

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

- A total of 203 FLAR cosses were programmed for AC distributed 13 crosses at the end of 1996, and 190 crosses on 1997.
- Of these crosses, 80 were processed yielding a total of 12,902 plants.
- The first set of doubled haploids plants generated from the crosses on '96 were selected both at Palmira Station for plant and grain type, and at Santa Rosa Experimental Station for blast resistance. Total of 81 doubled haploid lines were chosen and will be send to FLAR member countries for field evaluation.

The generation of doubled haploids is being used to advance breeding lines for FLAR. Sets of crosses which are selected by pedigree method, are also processed by anther culture. This way doubled haploids (DH) are used as an alternative to rapidly fix traits for the production of varieties or for the generation of improved parents from broad crosses. Two hundred and three triple crosses were selected for AC. F1 plants of 80 crosses were cultured. The selection of crosses is done based on their response to AC. The level of response is deduced from the pedigree or determined by a pre-test for culture response *in vitro*. The crosses are planted in the field, and plants to be cultured are pre-selected by plant and grain type, and age to maturity. Plants regenerated from AC (R1 plants) are then planted in the field, and doubled haploids plants are identified and selected for field trials. Results shown in Table 2 indicate that the total percentage of plants after the selection at Palmira and Santa Rosa is similar with doubled haploids and pedigree. Supporting the idea that the genetic variability recovered with doubled haploids is also similar to the pedigree population, with the difference that the traits in the DH are already fixed (alike to F6 plants), thus these materials are ready for field trials. In the first round of selection at Palmira , the number of DH plants were significantly reduced not only because of the selection for agronomic traits but also because only doubled haploids plants are selected from the R1 population. It is interesting to note, however, that three times as many DH plants were selected at Santa Rosa respect to pedigree, and it was possible to recover lines of three crosses which no lines were derived from pedigree (Table 2).

Collaborators: Daniel González (FLAR); James Gibbons (FLAR); Adriana Mora (IP-4); Zaida Lentini (IP-4, SB-2)

Table 2. Selection of doubled haploids F1-F2 plants from FLAR crosses at Palmira and Santa Rosa, 1997.

		DH selected				Plants selected by pedigree		
Cross	R1* plants	Palmira	Santa Rosa	Total Selection (%)	F1 plants	Palmira	Santa Rosa	Total Selection (%)
FL00001	952	54 (5.7)	13 (24.1)	1.4	72	37 (80.4)	8 (21.6)	17.4
FL00004	53	8 (15.1)	1 (12.5)	1.9	94	46 (48.9)	1 (2.2)	1.1
FL00005	417	37 (8.9)	9 (24.3)	2.2	163	49 (30.0)	3 (6.1)	1.8
FL00007	1946	118 (6.0)	23 (19.5)	1.2	201	52 (25.9)	5 (9.6)	2.5
FL00009	501	17 (3.4)	10 (58.8)	1.9	146	2 (1.4)	0	0
FL00012	706	77 (10.9)	20 (25.9)	2.8	80	0	0	0
FL00015	33	2 (6.1)	0	0	87	23 (26.4)	0	0
FL00016	215	14 (6.5)	5 (35.7)	2.3	76	0	0	0
Total	4833	327 (6.8)	81 (24.8)	1.7	919	209 (22.7)	17 (8.1)	1.8

Includes plants of various ploidies: haploids, diploids, and polyploids. Diploids (doubled-haploids) plants are first identified from the R1 population, and then selected for agronomic traits.
Numbers in parenthesis refer to the percentage of plants selected

5. Staff development and training

- Alfonso Sánchez. Instituto de Ciencias Nucleares y Energías Alternativas (INEA). Santa Fé de Bogotá, Colombia.
- Oneides Ovazoni. Instituto Rio Grandense do Arroz (IRGA). Porto Alegre, Brazil.
- Adriana Mora. B.Sc. Thesis. Universidad Santiago de Cali. Cali, Colombia.
- Frequent visits over the year by diverse B.Sc. and graduate students from different Universities of Colombia.

Collaborators: Adriana Mora (IP-4); Eddie Tabares (SB-2); Zaida Lentini (IP-4, SB-2)

OUTPUT 2. KNOWLEDGE OF THE PHYSIOLOGICAL BASIS FOR RICE TRAITS GAINED

A. NEW PLANT TYPE

JAMES GIBBONS (FLAR)

FLAR evaluated 446 new plant type selections at Santa Rosa in 1997. This architecture was designed as an improvement over the standard irrigated dwarf. Among the reported advantages of this plant are improved initial vigor, strong and thick culms, thick dark green foliage, large and full panicles, high harvest index, and low spikelet sterility. In general, initial vigor under our direct seeding conditions was poor. Eighty-six percent of the selections were rated as weak or very weak. Also, 75% were discarded due to high blast disease susceptibility. We selected, however, 31 lines for further testing (Table 1). These lines were selected for their large panicle size and strong culms. These lines will be included in the germplasm bank and/or distributed to countries.

Table 1. Characteristics of selected New Plant Type lines. FLAR, Santa Rosa, 1997.

Entry	Vigor(1)	Days to 50% Flow	Leaf Blast(2)	Neck Blast(2)	Temp Gel(3)	White Belly(4)	Grain Length(5)
IR70441-116-3	5	102	3	1	I/B	2.0	S
IR70441-150-1	7	89	2	3	BA	0.2	L
IR70441-150-2	5	92	4	1	A	0.6	L
IR70441-188-2	9	92	3	1	I	0.4	EL
IR70594-22-1	9	74	3	1	I	2.6	EL
IR70594-22-2	7	74	3	1	A	1.2	EL
IR71151-28	5	79	3	3	B,I	0.6	EL
IR71212-23	5	87	3	1	I	2.8	L

(1) Scale of 1-9, where 1 = extra vigorous, and 9 = very weak

(2) See Standard Evaluation System for Rice, IRRI

(3) A= High gel temp, I= Intermediate, B = low

(4) Scale of 1 to 5, where 1 is completely free of white belly, and 5 = totally chalky

(5) S = short grain, L = long , and EL = extra long grain

OUTPUT 2. KNOWLEDGE OF THE PHYSIOLOGICAL BASIS FOR RICE TRAITS GAINED

B. DEVELOPMENT OF RICE GERMPLASM TOLERANT TO SUBMERGENCE

Jaime Alberto Florez – Colciencias – CIAT – FLAR; James Gibbons - FLAR

Introduction

In Colombia and other regions of Latin America, weed control is heavily dependent on the application of chemical herbicides. This represents one of the highest costs to the producer. Red rice, which is the most troublesome weed in rice, is not controlled with rice herbicides. Weed control is greatly aided, however, once the permanent flood is established about one month to six weeks after seeding the rice. During the period of seedling establishment weeds compete with the rice, requiring herbicide applications. The repeated use of only one or two herbicide formulations has resulted in the development of herbicide resistant weed biotypes, and therefore more and heavier applications. Also, in the instances where good land leveling is not possible, low spots in the fields become flooded, resulting in poor rice germination and high weed populations. If the rice were able to germinate or grow rapidly under the water surface (anaerobically) this would help to reduce the emergence of weed seedlings and reduce seed mortality in the low spots, thereby aiding in the reduction of chemical herbicide usage.

Rice cultivars have been identified which posses the enzymatic activity necessary to grow under anaerobic conditions. These lines, however, are not adapted to Latin American conditions. It is necessary to find efficient methods to transfer these characteristics into adapted backgrounds.

Objectives:

- Identify evaluation methods for selecting genotypes with submergence tolerance.
- Identify tolerant and susceptible parents for use in a crossing program to introgress the trait or traits into adapted germplasm.
- Identify molecular markers closely linked to the gene or genes that confer tolerance to submergence to aid in selection of tolerant segregates.

Methods:

In the greenhouse, pregerminated seed (2-4 mm radical length) are planted under 10 cms. of water into puddled CIAT soil. Dry soil is added to cover the seeds approximately 3mm. The water level is maintained for 15 days after which evaluations are made according to the survival of the seedlings based on the IRRI evaluation scale.

We have obtained seeds of reported tolerant genotypes (Table1) from Asia which we are in the process of evaluating. These lines have many undesirable traits such as weak stems, colored grains, and disease susceptibility. We are hybridizing them with adapted Latin American germplasm. As of 1997 we have made 108 single crosses and have the F1 seed. We are in the process of obtaining markers which are closely linked to the *Sub1* QTL identified by Dr. David

Mackill USDA/ARS at the University of California, Davis, as accounting for 69% of the phenotypic variance for the trait in a cross between the tolerant IR40931-26 and susceptible PI543851. First we must confirm that the tolerant germplasm contains the *Sub1* QTL, then use those crosses with the gene to begin the backcrossing program to incorporate it into the adapted varieties.

Table 1. Rice germplasm reported as tolerant to submergence.

Variety	Origin
Karutha Vanan	Sri Lanka
JC 148	India
ASD 1	India
Taothabi	India
Rojofotsy 738	Madagascar
Guan Yin Tsan	China
Jawejan	Indonesia
Backoia	India
Awaria Katica	India
IR40931-26	IRRI

OUTPUT 3. RICE PESTS AND GENETICS OF RESISTANCE CHARACTERIZED

A. RICE PESTS AND GENETICS OF RESISTANCE CHARACTERIZED

FERNANDO CORREA (IP-4)

Executive Summary

Sustainable rice production is continuously threatened by the presence of diseases. Development and release of resistant cultivars to the most important pathogens in rice is the most effective alternative for the control of diseases. We have been focused in the characterization of the population structure of different rice pathogens as a means to understand their population dynamics and their interaction with the rice plant. Understanding changes in the pathogen population structure is a very important process for the identification of the most appropriate sources of resistance to be incorporated in a breeding program. Pathogen population studies are leading us to major benefits reducing breeding efforts and costs as well as having positive implications in the sustainability of rice production.

Several populations of the blast pathogen were characterized for their genetic structure and virulence diversity during 1997. Characterization of the blast pathogen changes overtime is leading us to the understanding of mechanisms leading to resistance breakdown of rice cultivars. We have been studying the resistance breakdown process in the highly blast resistant cultivar Oryzica Llanos 5 in Colombia since 1989. More than 200 blast isolates were characterized in 1997 for their genetic composition and pathogenicity on O. Llanos 5. Compatible isolates have been found under greenhouse studies, however a complete breakdown of the resistance is not occurring. Sources of resistance to this changing population of the blast pathogen have been identified both within exotic germplasm as well as local commercial rice cultivars. Compatible isolates were identified only within one genetic family of the pathogen. This genetic lineage has increased in frequency in the last two years. Analysis of the stable blast resistance to different genetic families of the blast pathogen in Oryzica Llanos is being conducted in 250 recombinant inbred lines. Results of greenhouse inoculations indicate the quantitative nature of the resistance. The biotechnology research unit is identifying molecular markers associated with major and minor resistance genes in O. Llanos 5.

Characterization and identification of resistance sources to leaf and panicle blast from different sources was conducted at the Santa Rosa experiment station. Results indicate that recurrent selection is a very effective method for breeding for blast resistance. Evaluation and selection of blast resistant rice lines in segregating populations should be conducted under proper field methodologies to reduce the occurrence of escapes. We have detected a high number of susceptible rice lines in trials that are close to cultivar release in different national programs. This indicate the lack of blast pressure followed by national programs in evaluating and selecting breeding populations.

Characterization of the blast resistance under greenhouse and field conditions of different rice germplasm has yielded a high number of lines exhibiting complementary resistance to the different genetic families of the blast pathogen. These lines are being used in genetic crosses aiming to combine the complementary resistance genes. Rice lines (F6) combining

the complementary resistance genes Pi-1 and Pi-2 exhibited a high level of resistance under high blast pressure at Santa Rosa experiment station as well as greenhouse evaluations. These two genes are being incorporated into commercial rice cultivars with desirable agronomic traits. Molecular markers are being used to follow the incorporation of the resistance genes Pi-1 and Pi-2 in different genetic backgrounds. Other simple and triples crosses have been made for combining complementary resistance sources to blast. Segregating populations are being evaluated for blast resistance.

We have confirmed the transmission of the rice entorchamiento virus by the vector *Polymyxa graminis*. Pure cultures of the fungus obtained from single cistosori were used in greenhouse inoculations to reproduce all entorchamiento symptoms. Using a fine root powder from infected plants as inoculum we developed a greenhouse screening methodology for identification of resistance sources. Several advanced rice lines exhibited high levels of resistance to entorchamiento and will be used in genetic crosses. Resistant rice lines contained roots infected by the fungus suggesting that resistance be only to the virus. Alternative control measures to reduce fungal populations are being identified in collaboration with other institutes. We have started to characterize populations of the brown spot pathogen in Colombia. A methodology for screening for resistance under greenhouse conditions is being developed. Incidence of the quarantine rice nematode, *Aphelenchoides besseyi* was determined in rice seeds of different Latin-American rice cultivars multiplied at CIAT. A hot water seed treatment is being implemented for the eradication of the nematode in the experiment station as well as in the outgoing seeds.

New techniques used in the characterization of blast pathogen populations are being transferred to national scientists in the region. Training is being offered at CIAT on the use of these new techniques for the development of durable blast resistance. Several publications as well as presentations at different national and international meetings were also used for the transfer of these new strategies. Graduate students from Cuba and Colombia are conducting their thesis work on blast research at CIAT.

Activity 1. Monitoring genetic and virulence diversity of the rice blast pathogen over time

Development of rice cultivars with blast resistance has been the preferred means to control the disease, however, resistance breakdown occurs normally in very short periods of time (1 to 3 years) after cultivar release. The great variability in virulence exhibited by the blast pathogen is associated with the quick breakdown of resistance. Understanding the population dynamics of the blast pathogen is a very important process in the development of breeding strategies for durable blast resistance. We have been studying the blast pathogen population structure over time in Colombia by characterizing race composition, virulence frequencies, and genetic structure using DNA fingerprinting. Monitoring changes within blast pathogen populations is a very important step for understanding plant pathogen interactions and mechanisms that might be leading to resistance breakdown as well as in identifying new sources of resistance to be used in genetic crosses.

Frequency studies of virulence and genetic families in the blast pathogen population in Colombia during 1997 revealed a reduction of lineageSRL-5 due to a reduction in the commercial area planted with the cultivar Cica 8. Lineage SRL-4 has increased as the susceptible cultivar Oryzica Caribe 8 has also increased in area planted with this cultivar. Monitoring the possible resistance breakdown of the cultivar Oryzica Llanos 5, which has exhibited a stable blast resistance under commercial conditions since 1989, continued during 1997. We reported in 1996 the occurrence of a higher incidence of blast lesions in O. Llanos 5. Genetic and virulence studies of isolates recovered from this cultivar indicated that isolates from lineage SRL-4 were recovered in higher frequency and were compatible with O. Llanos 5 in greenhouse inoculations. Analysis of isolates recovered from this cultivar in 1997 yielded again a large proportion of isolates from lineage SRL-4 (79%) while lineage SRL-2 and SRL-6 were recovered in lower frequency (Table 1).

Not all isolates from lineage SRL-4 recovered during 1996 and 1997 exhibited a compatible reaction with Oryzica Llanos 5 in greenhouse studies (Table 1). These isolates originated either from leaf or panicle infections in the field where Oryzica Llanos 5 exhibited a low leaf and panicle blast incidence and severity. Although we had speculated in the annual report of 1996 that a full breakdown of the resistance might happen during 1997, it did not occur. Oryzica Llanos 5 exhibited a maximum of 5-10% of leaf area affected, mainly as a result of the coalescence of small non-sporulating lesions (type 2 and 3). Isolates for greenhouse studies were recovered however from typical susceptible lesions in the leaf (type 4) or susceptible panicles. Incidence of susceptible panicles in the field is also very low (less than 5%), however the symptoms are fully typical and sporulating. No isolate from lineage SRL-2 or SRL-6 recovered from Oryzica Llanos 5 induced a compatible interaction with the cultivar at the leaf stage of 21 days old plants in greenhouse inoculations (Table 1).

Panicle blast is more important than leaf blast, however, most studies do not consider panicle evaluations as leaf and panicle reactions are normally under the same genetic control. Fourteen blast isolates recovered from infected leaves or panicles of O. Llanos 5 were used for greenhouse inoculations at the panicle stage in the greenhouse (Table 2). These isolates were from lineage SRL-2, SRL-4 and SRL-6, which exhibited or not a compatible interaction with Oryzica Llanos 5 at the leaf stage in the greenhouse (Table 2). All the isolates induced a fully susceptible reaction with the cultivar when inoculated in the panicle following a panicle injection method recommended for international panicle blast resistance testing (Table 2). It is possible that the recommended method for inoculation is too severe inducing a susceptible reaction of the inoculated panicles. We will continue developing more studies on panicle inoculations (different inoculation methods) and evaluations to clarify and understand the correlation between leaf and panicle reaction. We need to understand what is the meaning of this susceptible reaction at the panicle stage in the greenhouse and its correlation with field reaction. Inoculations need to be performed with those genetic lineages (SRL-1, SRL-3, SRL-5) of the pathogen, which have not been recovered from infected samples. Results from panicle experiments will be very important in understanding resistance breakdown mechanisms and will have implications in the selection of sources of resistance for genetic crosses.

Identification of new variants or virulent forms within a pathogen population is very important for the selection of potential sources of resistance against them. The virulence spectrum of blast isolates compatible with Oryzica Llanos 5 is shown in Table 3. These isolates have a wide spectrum of virulence, however several genes confer resistance to them (Table 3). These isolates did not infect both, genes Pi-1 and Pi-2, which are being used in combination in this project as a means to protect against all genetic lineages of the blast pathogen. The resistance gene Pi-k, probably at the same locus as Pi-1, confer resistance to this lineage (Table 3). Oryzica Llanos 5 exhibits a low disease severity, suggesting that its resistance is still highly effective (Table 3). We will continue monitoring the blast population recovered from this cultivar to determine the real relevance of these isolates for the resistance breakdown of O. Llanos 5. We will look at the adaptability of these isolates in the field and measure some of the fitness components, such as sporulation capacity, infection efficiency in laboratory and greenhouse studies.

Activity 2. Evaluation and characterization of rice germplasm for blast resistance

Evaluation and characterization of rice germplasm for leaf and neck blast reactions are conducted on an annual base at the Santa Rosa experiment station in the department of Meta, Colombia. More than 700 lines from different origin (local and introduced) were evaluated under severe blast pressure in the field in 1997 (Table 4). A high blast pressure is given by using spreader rows composed of a mixture of rice cultivars susceptible to the different genetic lineages of the blast pathogen in Colombia. New sources of resistance, exhibiting a complete or partial resistance are selected for continuo evaluation and characterization over time, including greenhouse inoculations. A population derived from a recurrent selection project for blast and with the additional purpose of broadening the genetic base yielded the highest number of resistant lines to blast (Table 4). These lines will be distributed to different national programs in the region to be included as sources of resistance in their breeding programs.

CIAT has changed its breeding priorities, activities, and relations with national programs in the last two years. We are moving from the concept of distributing fixed or advanced lines to the distribution of segregating populations and potential sources of different traits of well-characterized germplasm. A potential weakness of this strategy for blast might be the lack of proper field methodologies followed by national programs for the evaluation and selection of true blast resistant lines within those populations. Two rice cultivars, Oryzica Caribe 8 and Oryzica Yacu 9, which were released in Colombia after Oryzica Llanos 5, turned susceptible after one year of their release. These cultivars were selected from segregating populations given to national researchers. Characterization of the blast reaction of rice lines that have reached advanced stages in national programs after their own selections was conducted at Santa Rosa in 1997 (Table 4). These nurseries included advanced lines, promising lines, regional trials, and efficiency trials or prueba de eficiencia. Most of these lines exhibited a susceptible reaction to blast (Table 4). These results demonstrate the need of a proper field methodology and selection of a good screening site such as the "hot spot" used by the rice project from CIAT at Santa Rosa. These aspects need to be discussed with national programs during future meetings if true blast resistant cultivars are to continue being released.

A very broad spectrum of virulence, expressing compatibility with all resistance genes reported in the literature, has been found in blast populations analyzed at the Santa Rosa experiment station and reported previously. We have been using a group of isolates representing the six more important genetic families of the blast pathogen in Colombia (SRL1 to SRL-6) for identifying potential sources of blast resistance for the Latin American region under greenhouse inoculations. As a group, these isolates express virulence on most resistance genes. Sources of resistance to the different genetic families of the blast pathogen were identified in 1997 in greenhouse inoculations working in collaboration with FLAR (Table 5). More than 300 rice lines from the germplasm bank were characterized for their blast reaction to 12 isolates of the pathogen representing the six genetic families in the greenhouse. Rice lines exhibiting a complete or complementary resistance to the different genetic families were identified and are being used by FLAR in genetic crosses. These sources of resistance will continue being characterized for the stability of their reaction in the greenhouse and field conditions. Genetic lineage SRL-6, the most predominant lineage in nature in Colombia, was the most virulent to the rice germplasm tested (Table 5). More resistance genes to this lineage need to be identified and incorporated in crosses to reduce the frequency of this blast population.

Activity 3. Improving breeding methods for developing durable blast resistance

One of the most important activities developed in this project is related to the development of a breeding strategy that allows to use rice cultivars with desirable agronomic characteristics (yield, quality, etc) but susceptible to blast. This strategy is based on the identification of rice lines, which are susceptible to only part of the pathogen population (one or two genetic families of the pathogen), but with resistance genes to the rest of the population. Crosses and development of rice populations between these rice lines with complementary resistance to blast (resistance to different genetic families) have allowed us together with researchers at IRRI and Purdue University, to develop the lineage exclusion hypothesis. These crosses give origin to rice lines combining complementary resistance genes that exclude all possible compatible interactions with the entire blast population at a particular site or region. Accumulation of the resistance genes Pi-1 and Pi-2 in single rice lines confers resistance to the entire blast population in Colombia, both in the field as well as in greenhouse inoculations during 1997 (Table 6). Although different genetic families of the blast pathogen (Table 6) defeat each individual resistance gene, there is no single isolate compatible with both genes. The best fourteen F6 rice lines selected during 1997 (Table 6) from the cross C101A51 (Pi-2) x C101LAC (Pi-1) will be tested under different conditions for corroboration of their blast resistance. Presence of the two resistance genes in rice lines derived from this cross is being conducted in collaboration with the biotechnology unit at CIAT. The resistance gene Pi-2 is being followed with the molecular marker SCAR B-10 (Figure 1) developed from a RAPD marker at the biotechnology unit while Pi-1 is followed by other RFLP markers developed elsewhere. Rice populations developed with the same two genes or other rice lines in those collaborating institutions as well as CIAT, are being exchanged for comparisons with different blast populations. These lines will be used at CIAT in genetic crosses for incorporation of the two genes in rice cultivars with desirable agronomic traits that are susceptible to blast. Some of these commercial cultivars are: Oryzica 1 and Cíca 8 in Colombia, IRGA 409 in Brasil, El Paso 144 in Uruguay and Argentina, Rustic in Guyana, J 104 in Cuba, IR 50 in India, etc.

More than 200 commercial rice cultivars from Latin America were characrterized for their blast reaction to different genetic of the blast pathogen in Colombia during 1996 and 1997.

Several of these cultivars exhibited complementary resistance to two or three genetic families and simple and triple crosses among these cultivars (Table 7) were realized to exclude all the compatible genetic families of the pathogen. Segregant populations of these crosses are being tested under field conditions and resistant rice lines selected for greenhouse inoculations. Several hundred of rice lines have been evaluated and selected by FLAR under the same criteria in 1997. Segregant populations of these crosses are being tested for their blast resistance in Santa Rosa.

Activity 4. Analysis of the genetics of resistance and dissection of blast resistance genes in highly resistant cultivars

We have started to characterize the quantitative nature of the stable blast resistant rice cultivar Oryzica Llanos 5 in 1997. Analysis of the reaction of F2 segregating lines, and 250 recombinant inbred lines (RIL's) of the cross Oryzica Llanos 5 (resistant) / Fanny (susceptible) suggest the complexity of such cultivar (Table 8). The quantitative and continuous expression of the blast reaction (percentage leaf area affected) observed in the 250 RIL's to eight isolates of the different genetic families of the pathogen (Table 8) used in greenhouse inoculations suggest the presence of major and minor resistance genes in Oryzica Llanos 5. This pattern of reaction is mainly observed with isolates from genetic lineage SRL-6 and SRL-4 to which a less number of resistant lines were detected (Table 8). The cultivar Oryzica Llanos 5 was highly resistant to all blast isolates used exhibiting only lesions type 1. The cultivar Fanny was highly susceptible to all isolates except to lineage SRL-2 (9% leaf area affected). Most isolates used in the inoculations differed in their spectrum of virulence (Table 8), and genetic lineage SRL-3 was the least virulent. We will continue inoculating in the greenhouse these populations as well as F3 lines during 1998.

These populations and results of their blast reactions to the different genetic families of the fungus are being used by the biotechnology unit for the identification of molecular markers (RFLP's, RAPD's, and microsatelites) associated with the genes controlling the resistance in the cultivar Oryzica Llanos 5. Progress on the identification of molecular markers linked to some of the resistance genes in Oryzica Llanos 5 will be reported in project SB-2. Molecular markers will be used for following the introgression of resistance genes controlling durable blast resistance into different genetic backgrounds.

Activity 5. Evaluation and characterization of rice germplasm for resistance to *Tagosodes oryzicolus* and the rice hoja blanca virus (RHBV)

Results of this activity are being reported within this IP-4 project by Dr. Lee Calvert.

Activity 6. Isolation and characterization of the fungal vector (*Polymyxa graminis*) of rice entorchamiento virus (rice stripe necrosis virus)

Entorchamiento or rice stripe necrosis virus has increased in incidence and severity reducing yields in different rice growing areas in Colombia. Individual cistosori of the vector fungus were obtained from infected roots under a microstereoscope and inoculated into healthy roots of 3 days old seedlings. Pure isolates of the vector *Polymyxa graminis* were obtained after repeating two times the inoculations with individual cistosori isolated from infected roots in the greenhouse. All entorchamiento symptoms were reproduced in plants of the rice cultivar Oryzica 3 inoculated with the vector *P. graminis.* Symptoms developed were twisting, malformations, stunting, yellowing, stripe necrosis, and plant death. Virus particles of RSNV were observed in the electron microscope. Most roots of infected plants contained cistosori of the fungal vector.

A greenhouse inoculation method was developed for screening rice germplasm for resistance to entorchamiento. Fine powder containing cistosori was prepared from infected roots of the cultivar Oryzica 3 inoculated with pure cultures of the fungus and incorporated in small plastic pots containing sterile sand. Infected fine powder can be stored at low temperature for long periods of time and used for several inoculations. Five to ten seeds of different rice cultivar/lines were planted per plastic pot containing the cistosori. Incidence and severity of entorchamiento increase using higher concentrations of cistosori in the plastic pots. Good symptoms were observed with a concentration of 100000 cistosori per gram of powder and mixing at least 0,10 grams of root powder per pot. Symptom development depend on the susceptibility of the cultivar. Resistant cultivars exhibited symptoms at 80 days after inoculation while symptoms were observed after 15 days in susceptible cultivars.

Potential sources of resistance to the virus were observed in different rice line/cultivars in inoculations with infected root powder in the greenhouse (Table 9). Most of the commercial rice cultivars exhibited a highly susceptible reaction. The best commercial cultivars in several inoculations were Oryzica Yacu 9 and Linea 2. The rice line CT 11026-3-9-1t-2p-2p-2 did not exhibit any symptom in two repeated trials. The rice cultivar Makalioka has exhibited a high level of resistance in more than five different trials (Table 9). Observations of roots collected from resistant cultivars/lines have shown presence of high number of cistosori of *P. Graminis*, suggesting that resistance is to the virus and not to the fungus. Inoculation of the susceptible cultivar Oryzica 3 with powder containing cistosori produced in roots of resistant cultivars has reproduced the symptoms of entorchamiento. More trials will be conducted in 1998 to determine if virus concentration will be reduced in time in cistosori collected from resistant cultivars or rotation crops. Other studies on the epidemiology, control measures, and identification of potential sources of resistance will continue during 1998. Resistance of rice lines identified in these studies will be proved under field conditions in trials with CORPOICA and FEDEARROZ.

Activity 7. Isolation and characterization of the rice brown spot ("Helminthosporium disease") pathogen (*Bipolaris oryzae*)

A rice brown spot epidemic was observed during the second planting season in several rice growing areas of Colombia in 1996. The most affected commercial rice cultivar was Oryzica Yacu 9, for which some farmers in the Tolima area reported up to 40-50 % yield losses in their crops. The disease appeared in the field as early as 30 days after planting in this cultivar and was not associated with nutrient deficiencies or sandy soils. Other cultivars are normally attacked late in the season, exhibiting brown spot symptoms 80-100 days after planting. We visited farmer fields together with researchers from FEDEARROZ, ICA, and CORPOICA to recognize the problem in the area. Several actions were taken by each institution after having met at FEDEARROZ in Tolima. Infected leaf samples were

collected to initiate studies on the isolation, identification, multiplication, and inoculation of the brown spot pathogen at CIAT. . Isolates of the fungus were obtained in the laboratory after induction of sporulation of the pathogen in infected samples.
Morphological comparisons and growth on artificial media of isolates recovered from the cultivar O. Yacu 9 and other rice commercial cultivars did not show any difference. Results obtained here suggest that the epidemic observed in 1996 is probably due to the susceptibility of the cultivar O. Yacu 9 favored by higher temperatures and not to the presence of a different fungal species.

Greenhouse inoculations of several commercial cultivars showed high compatibility with the fungus independently of the origin of the isolate. Better symptoms of brown spot and differences in reaction among commercial rice cultivars were observed in inoculations of 70-90 days old plants than 25-40. We will continue developing a greenhouse methodology for testing and identifying potential sources of resistance to brown spot. Resistant lines will be tested in collaboration with FLAR in the field in Colombia as well as potential hot spot areas existing in Panama.

Activity 8. Evaluation of the incidence and control of the rice nematode of quarantine importance (*Aphelenchoides besseyi*) in CIAT rice fields

The nematode *Aphelenchoides besseyi* , causal agent of the white tip disease in rice, is frequently detected on rice seeds of healthy looking plants in multiplication fields at CIAT. Seed transmission is the main source of inoculum as well as of distribution of the pathogen to other countries. Eradicating the nematode from infected seeds can control the disease. A total of 214 rice cultivars from 26 countries in Latin-America were harvested at CIAT and examined for the presence of the nematode. The percentage of infection was between 0 and 55%. Thirty cultivars did not carry any nematode. The number of nematodes per seed was between 1 and 38 nematodes. Rice cultivars from Chile had the highest percentage of seed infection (25.5%) while rice cultivars from the USA had the lowest (4.4%). Rice cultivars from Colombia had in average 12.4% infection.

Seeds of the ten more infected rice cultivars were selected and treated with hot water at different temperatures to control the nematode. Heat treatment was at the temperatures of 55, 60, 62, 63 and 65 C for 15 minutes (Table 10). The treatments at 62, 63, and 65 controlled 100% of the nematodes in the seeds. The percentage of infected seeds treated at 60C was between 0-2 % and 2 to 14 % when treated at 55C (Table 10). Treatment at fifty-five C is internationally recommended for controlling the nematode in rice seeds. This treatment was only partially effective in our experiments (Table 10). Only the heat treatment at 65C reduced significantly seed germination in 3 out of 10 rice cultivars treated. Chemical control using recommended doses and products did not control the nematodes in the seeds efficiently. We are recommending realizing heat treatments at 62C for 15 minutes to eradicate nematodes in rice seeds going to multiplication fields or outgoing shipments.

Activity 9. Elucidation of the rice root syndrome (sindrome de las raices negras) present in farmer fields in Venezuela

The sindrome de las raices negras in Venezuela is a serious complex of biotic and abiotic factors affecting rice production in Venezuela for several years. Affected plants usually show normal growth and color in early stages of development, with the first symptoms being a change in root color from white or light brown to black. The whole root system becomes black and begins to deteriorate. In advanced stages of the disease there is a bad odor in infected fields. Chemical and physical disorders have been associated to the problem after several visits and analysis of soil scientists. We have examined the association of root pathogens to the problem and have isolated mainly *Phytium* spp., which is a common root pathogen in rice. Isolates were identified within this specie using specific primers for PCR analysis. Inoculations of healthy roots under greenhouse conditions have been negative for reproducing the symptoms observed in infected fields with sindrome de las raices negras. It is probably that *Phytium* is acting only as a secondary factor after roots start to deteriorate.

A very similar problem in rice has been reported in Japan and California's rice fields. The disease is known as Akiochi (hydrogen sulfide toxicity). The problem is caused by high amounts of hydrogen sulfide found in soils relatively high in organic matter and soluble sulfates that become highly reduced after flooding. Rice fields are continuously planted after harvesting in Venezuela not given enough time for decomposition of plant residues. Fields are planted two and a half to three times per year with no crop rotation. Fields are flooded most of the year with very short periods of dryness between planting seasons in Venezuela. Hydrogen sulfide gradually accumulates in California flooded soils, especially where there is little active Fe to precipitate the sulfide ion.

The best control of the problem seems to be the draining of affected fields to supply oxygen to affected plants. Several alternatives of control are being implemented in Venezuela to solve the problem.

Activity 10. Training of Latin-American rice scientists in new rice pathology techniques

A project to study the blast pathogen population in Cuba was initiated in 1996 and continued during 1997. One scientist trained in 1996 at CIAT came back to initiate his Ph.D. work on the characterization of the genetic structure of the blast pathogen population in Cuba. Infected blast samples were collected from different rice cultivars and regions in Cuba for the analysis. Virulence diversity studies of the same blast population will be conducted when he goes back to his country. Blast incidence and severity has increased in that country in the last three years affecting yields severely. Genetic diversity of Cuban rice materials will also be analyzed in his work in collaboration with the biotechnology unit.

Two rice researcher from Brasil have been trained for a period of two months on the characterization of the virulence diversity of the blast pathogen and the use of molecular markers for characterizing the genetic structure of the fungus. Infected blast samples were collected from different areas and cultivars and Brasil for analysis. Analyses of the autorads generated during the training period are being analyzed at this moment. The same populations will be analyzed in grenhouse inoculations in Brasil for determining the virulence diversity.

A Colombian Ph.D. student and professor from the Unviersidad Nacional has finished his course work and will start his field work early in 1998. He will work on the characterization of blast populations from different hosts and rice species in different ecosystems in Colombia. His main objective is to determine the role and mechanisms of these populations in the generation of pathogenic diversity in the rice blast pathogen. A Ph.D. student from the USA, University of Florida has finished his field work on the use of silicon for the control of rice diseases and improvement of yields in the acid soils of the Colombian Llanos. He will be graduating early in 1998.

Table 1. Genetic lineage of blast isolates recovered from oryzica llanos 5 and oryzica caribe 8 and pathogenicity on oryzica llanos 5 in greenhouse inoculations

ORIGIN OF ISOLATE	YEAR	TISSUE	No. Isolates	GENETIC LINEAGE SRL-4	SRL-6	SRL-2	ND
O.LLANOS 5	1996	LEAF	35	25 (17+)	3	5	2
		NECK	28	10 (4+)	2	15	1
	1997	LEAF	46	35 (1+)		3	8
		NECK	42	21 (4+)	5	2	14
O.CARIBE 8	1996	LEAF	42	36 (26+)	1		5
	1997	LEAF	7	3 (2+)			4
		NECK	34	14 (2+)	2		18
TOTAL (Virulent)			234	144 (56+)	13	25	52

ND= Not determined

Table 2. Leaf and panicle blast reactions of rice cultivar oryzica llanos 5 in greenhouse inoculations

			BLAST REACTION	
	ORIGIN	GENETIC	LEAF	NECK
ISOLATE	ISOLATE	LINEAGE	(0-9)	(0-9)
O. LLANOS 5 (208-1)	LEAF	SRL-4	4	7-9
O. LLANOS 5 (212-1)	LEAF	SRL-4	4	9
O. LLANOS 5 (236-1)	LEAF	SRL-4	4	5-9
O. LLANOS 5 (239-2)	LEAF	SRL-6	0	7-9
O. LLANOS 5 (240-1)	LEAF	SRL-4	4	7-9
O. LLANOS 5 (243-1)	LEAF	SRL-4	4	3-9
O. LLANOS 5 (245-1)	LEAF	SRL-4	3,4	7-9
O. LLANOS 5 (249-1)	NECK	SRL-2	0	7-9
O. LLANOS 5 (259-1)	NECK	SRL-4	0	5-9
O. LLANOS 5 (262-1)	NECK	SRL-4	3,4	7-9
O. LLANOS 5 (264-1)	NECK	SRL-6	0	9
O. LLANOS 5 (271-1)	NECK	SRL-2	0	7-9
O. LLANOS 5 (272-1)	NECK	SRL-4	0	7-9
O. LLANOS 5 (274-1)	NECK	SRL-4	4	5-9

Greenhouse inoculation: Leaf = spraying spore suspension; neck: injection
Blast reaction: 0= highly resistant; 9= highly susceptible

Table 3. Virulence spectrum of genetic lineage srl-4 recovered from oryzica llanos 5 and identification of sources of resistance

Cultivar	Resistance gene	Lesion Type	LAA %	Cultivar	Lesion type	LAA %
Aichi Asahi	Pi-a	4	55	Metica 1	4	12
BL-1	Pi-b	4	13	Oryzica 1	4	12
Caloro	Pi-ks	4	29	Oryzica 2	0	0
Chokoto	Pi-a,Pi-k	0	0	Oryzica 3	0	0
Dular	Pi-ka	4	7	CICA 4	4	1
Fukunishiki	Pi-z	4	13	CICA 6	0	0
Fujisaka 5	Pi-i,Pi-ks	4	13	CICA 7	4	16
IR 42		4	3	CICA 9	0	0
Kanto 51	Pi-k	0	0	IR 22	0	0
Kusabue	Pi-k	0	0	CICA 8	0	0
K 1	Pi-ta	4	11	IR 8	0	0
K 59	Pi-t	0	0	Tetep (Pi-k)	0	0
NP- 125		2	4	Ceysvoni	2	10
Pi. No 4	Pi-ta2	4	9	O. Llanos 5	4	5
Ramin. Str 3		4	6	Linea 2	4	5
Sha tiao tsao	Pi-ks	4	82	O. Llanos 4	4	1
Tsuyuake	Pi-km,Pi-m	1	1	IRAT 13	0	0
Usen	Pi-a	4	8	Moroberekan	0	0
Zenith	Pi-z,Pi-i	4	26	O. Sabana 6	13	1
Bluebonnet	Pi-a	3	8	O. Caribe 8	4	8
C101 A51	Pi-2	4	60	O. Yacu 9	1	1
C101 LAC	Pi-1	2	4	Selecta 3-20	4	11
C101 PKT	Pi-4a	4	12	Colombia 1	4	3
C104 PKT	Pi-3	4	60	Fanny	4	100
C105 TTP-4	Pi-4b	4	39			

LAA= Leaf area affected; Lesion type: 0-3=resistant; 4= susceptible

Table 4. Evaluation and characterization of rice germplasm for leaf and neck blast reactions at santa rosa experiment station, 1997

ORIGIN	NUMBER	BLAST REACTION (LEAF/NECK)			
NURSERY	LINES	(1-3)	(4)	(5)	(>5)
		Number of lines			
PARENTS RECURRENT SELECTION	30	8	11	8	3
RECURRENT SELECTION (C2P1)	58	53	3	2	
RECURRRENT SELECTION (C2P3)	31	21	4	5	1
URRN (USA 96)	51	38	7	6	
URRN (USA 97)	199	1	7	45	146
GERMPLASM BANK (CIAT)	29	6	3	9	11
BG 90-2 x O. Rufipogum	140	29	4	53	54
FLAR LINES (Promisorias)	33	4	1	10	18
FEDEARROZ (Advanced lines)	15	4		5	6
IRGA (High yield)	23				23
VENEZUELA (Advanced lines)	36	15	3	11	7
FLAR (R2)	80	9	4	13	54
FEDEARROZ (Regional trial)	9		1	2	6
FEDEARROZ (Prueba eficiencia)	10	2	6	1	1
TOTAL	744	190	54	170	330
Percentage (%)		25.5	7.3	22.8	44.4

Blast reactions: (1-3)= resistant; (4)= intermediate; (5)= susceptible; (>5)=highly susceptible

Table 5. Blast reaction of 300 rice lines evaluated for flar with 12 isolates of six genetic lineages of *pyricularia grisea* in the greenhouse

GENETIC LINEAGE	RESISTANT (%)	INTERMED/SUSCEPTIBLE (%)
SRL-6	133 (44)	167 (56)
SRL-5	245 (82)	55 (18)
SRL-4	248 (83)	52 (17)
SRL-3	297 (99)	3 (1)
SRL-3	270 (90)	30 (10)
SRL-1	268 (89)	32 (11)

Table 6. Rice lines (f6) from the cross c101 lac (pi-1) x c101 a51 (pi-2) resistant to blast in the field and greenhouse inoculations

RICE LINES	FIELD REACTION		GREENHOUSE	
	LEAF	NECK	SRL	SRL
	(1-9)	(1-9)	6/4/2/1	5
CT 13432 (PL2)-1-1-M-M-M	2	1	R	R
CT 13432 (PL2)-4-2-M-M-M	2	3	R	R
CT 13432 (PL2)-11-1-M-M-M	3	3	R	R
CT 13432 (PL4)-2-1-M-M-M	3	3	R	R
CT 13432 (PL4)-2-2-M-M-M	3	3	R	R
CT 13432 (PL4)-14-1-M-M-M	3	1	R	R
CT 13432 (PL5)-1-1-M-M-M	2	1	R	R
CT 13432 (PL5) -1-2-M-M-M	2	1	R	R
CT 13432 (PL7)-5-2-M-M-M	3	3	R	R
CT 13432 (PL7)-7-1-M-M-M	3	3	R	R
CT 13432`(PL7)-10-1-M-M-M	2	3	R	R
CT 13432 (PL8)-7-2-M-M-M	2	1	R	R
CT 13432 (PL8)-9-1-M-M-M	2	3	R	R
CT 13432 (PL8)-10-2-M-M-M	3	3	R	R
CT 13432 (PL8)-15-3-M-M-M	2	1	R	R
C101 A51 (Pi-2)	8	9	S (9)	R (1)
C101 LAC (Pi-1)	5	5	R (1)	S (7)

R=resistant (1-3); S=susceptible (>4). SRL= Blast Genetic Lineage 1 to 6

Table 7. Simple and triple crosses combining complementary resistance sources to colombian blast populations

CROSS TYPE	GENETIC CROSS	COMPATIBLE GENETIC FAMILIES
SIMPLE	ORYZICA CARIBE 8 / ORYZICA YACU 9	SRL-4, SRL-6
SIMPLE	ORYZICA CARIBE 8 / PERLA	SRL-4, SRL-5
SIMPLE	ORYZICA CARIBE 8 / PA3	SRL-4, SRL-6
SIMPLE	PERLA / ORYZICA YACU 9	SRL-5, SRL-6
SIMPLE	ORYZICA CARIBE 8 / AMISTAD 82	SRL-4, SRL-5
TRIPLE	ORYZICA CARIBE 8 / PERLA // ORYZICA 3	SRL-4, SRL-5, SRL-6
TRIPLE	ORYZICA CARIBE 8 / AMISTAD 82 // O. YACU 9	SRL-4, SRL-5, SRL-6

SRL= Genetic families of the blast pathogen in Colombia

Table 8. Leaf blast reaction of 250 recombinant inbred lines (ril's) of the cross oryzica llanos 5 x fanny in the greenhouse

		BLAST GENETIC LINEAGES							
LAA	Lesion	SRL-6	SRL-6	SRL-6	SRL-5	SRL-4	SRL-3	SRL-2	SRL-1
%	Type	Iso 1	Iso 2	Iso 3	Iso 1	Iso 1	Iso 1	Iso 1	Iso 1
		Number of lines							
0	0-2	66	172	121	162	21	170	171	167
1-10	3	63	13	31	36	36	39	43	26
1-5	4	31	14	23	29	83	10	32	27
6-10	4	23	11	15	7	44	9	1	3
11-20	4	22	7	17	7	40	7	2	6
21-30	4	14	7	5	4	14	2	0	3
31-50	4	13	12	15	3	12	2	1	5
>50	4	16	12	20	2	3	9	0	11
Cross		Leaf Area Affected (%)							
Fanny	4	85	100	88	55	60	100	9	100
OLL5	0-1	R	R	R	R	R	R	R	R
Resist Gene		Leaf Blast Reaction							
Pi-1	4				+				
Pi-2	4	+	+			+		+	+
Pi-3	4	+	+		+	+			
Pi-4a	4	+	+	+	+	+			
Pi-4b	4	+	+	+	+	+			

Lesion Type: 0-2=resistant; 3=intermediate; 4=susceptible. LAA=leaf area affected.
Iso= blast isolate. += compatible interaction. R=resistant

Table 9. Incidence of "entorchamiento" or rice stripe necrosis virus in rice germplasm inoculated in the greenhouse with the vector *polymyxa graminis*

CULTIVAR/LINE	ENTORCHAMIENTO %	STRIPE SYMPTOMS %
CT 11026-3-9-1T-2P-2P-2	0	0
MAKALIOKA	0-20	0-20
CT 11032-2-4-3T-3P-3P-1	7	7
CT 10323-8-2-2P-1-1T-4P-4P	7	7
CT 10491-12-4-2T-3P-1P-3	13	13
COLOMBIA 3	20-30	20-30
COLOMBIA 1	20-47	20-53
COLOMBIA 2	40	10-40
MUDGO	7-10	7-10
ORYZICA YACU 9	7-40	0-50
ORYZICA CARIBE 8	20-27	30-40
ORYZICA 1	7-50	7-50
ORYZICA 3	67-73	67-73
ORYZICA LLANOS 5	90	90
LINEA 2	20	20
CICA 8	50	50

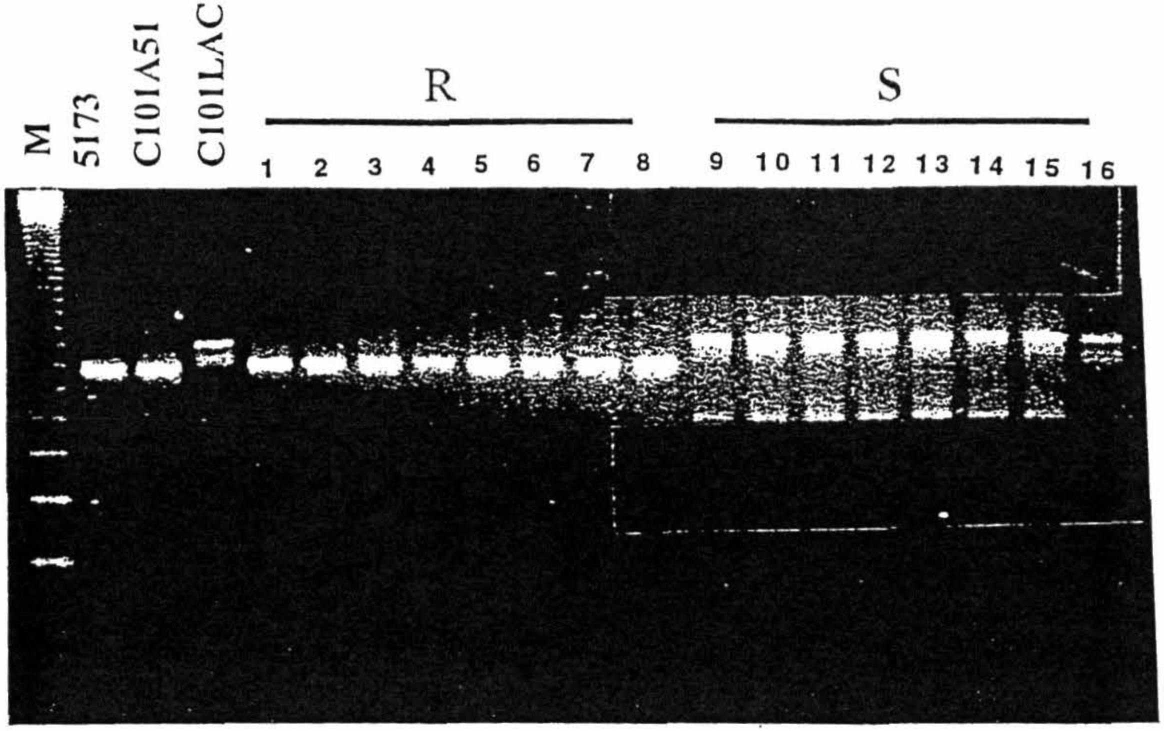
Range of disease given for those cultivars included in three diiferent trials

Table 10. Heat treatment to erradicate the nematode *aphelenchoides besseyi* from infected rice seeds

HEAT TREATMENT T EMPERATURE 15 min	SEEDS WITH NEMATODES %	SEEDS WITH ALIVE NEMATODES (%)	GERMINATION AFTER TREAT. %
CHECK	28 - 55	28 - 55	84 - 98
55 C	26 - 39	2 - 14	83 - 100
60 C	32 - 45	0 - 2	54 - 98
62 C	29 - 45	0	79 - 98
63 C	28 - 44	0	52 - 97
65 C	30 - 49	0	27 - 96

Average of 10 cultivars. Ninety six seeds/cultivar/treatment

SCAR B-10 F5 (pi-1 x pi-2)



1	CT13432(PL2)-1-1-M	4	CT13432(PL2)-11-1-M	2	CT13432(PL2)-4-2-M	3	CT13432(PL2)-9-1-M
8	CT13432(PL4)-14-1-M	5	CT13432(PL4)-2-1-M	6	CT13432(PL4)-5-1-M	7	CT13432(PL4)-6-2-M
9	CT13432(PL5)-1-1-M	10	CT13432(PL5)-2-1-M	11	CT13432(PL5)-2-2-M	13	CT13432(PL6)-12-1-M
14	CT13432(PL6)-13-2-M	12	CT13432(PL5)-7-2-M	16	CT13432(PL7)-5-2-M	17	CT13432(PL8)-7-2-M

Figure 1

B. CONTROL OF RHBV THROUGH NUCLEAR PROTEIN MEDIATED CROSS PROTECTION AND ANTI-SENSE RNA STRATEGIES. (PROJECTS IP4 & SB2)

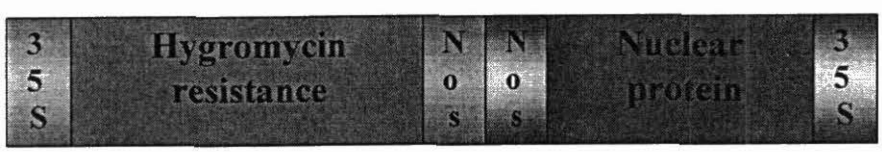
1. Preparation of plasmid constructions containing the RHBV nuclear protein gene.

- A plasmid construction containing the N-gene was modified to allow linearized plasmid to be used in the ballistic bombardment of rice tissue.

The plasmid used for introgression of the nuclear protein gene (N-gene) of RHBV into rice is a combination of two genes. The hph-gene that confers hygromycin resistance was flanked using the CaMV 35S promoter and the Nos polyadenylation sequence. A similar construction was made for the RHBV N-gene and the gene cassettes were joined into a single plasmid. The gene cassettes were oriented so that the RNA transcription of each gene was in opposite directions (figure 1). This plasmid was named RHBV-NP. The original experiments were made using circular plasmid. Later experiments used this same plasmid construction with minor modifications to remove some of the poly-linker restriction enzyme sites. This allowed the plasmid to be linearized.

Collaborators: Iván Lozano (IP4); Lee Calvert (IP4)

Figure 1. A diagram of the construction of the RHBV N-gene that was used to produce transgene CICA 8 rice that is resistant to RHBV



2. Transform rice with RHBV-NP constructs.

- A total of 187 Cica 8 plants from the RHBV-NP circular plasmid bombardments were obtained.
- Sixty of the 187 (32%) plants recovered from the RHBV-NP experiments using circular plasmid, contain the RHBV N-gene.
- In all cases, NP-fragments larger than the expected size were visualized on the Southern blots suggesting the presence of rearrangements.
- Current experiments include the use of linear expression vectors.
- About sixty BR-IRGA 409 plants have been recovered from RHBV-NP linear plasmid bombardments.
- When the linear plasmid construct was used, most of the plants showed the expected N-gene size fragment, as well as fewer integration sites and rearrangements were also noted.

The direct delivery of genes into immature embryos or immature panicle-derived callus was conducted using DNA-coated gold particles accelerated by the PDS-1000/He system. The tropical irrigated Latin American *indica* varieties Oryzica 1, Cica 8, and Inti and the tropical upland japonica line CT 6241-17-1-5-1 were used as targets. Either circular or linear plasmid forms of the RHBV-NP constructs were used. The hygromycin resistance conferred by the hph-gene was used as the selective marker. The putative transgenic events were recovered using a step-wise selection on culture medium containing 30 mg/l hygromycin B (hyg B) followed by 50 mg/l hyg B throughout plant regeneration. The efficiency of this transformation protocol was genotype dependent and from 2 to 33 explants were initailly bombarded to obtain hygromycin resistant plants. The transformation protocol After the complete step-wise selection process throughout plant regeneration on 50 mg/l hyg B, a total of 187 plants from the RHBV-NP circular plasmid bombardments were obtained. Sixty of the 187 (32%) plants were recovered from the RHBV-NP experiments using circular plasmid, contain the RHBV N-gene. In all cases, the bands visualized on the Southern blots were larger than the expected N-gene plasmid construction suggesting the presence of rearrangements (Figure 3). Apparently, a variety of integration patterns had been obtained in other laboratories especially when circular plasmid is used. Current experiments include the use of linear expression vectors. Larger number of plants with the expected NP gene size fragment, fewer integration sites and rearrangements were noted when linear plasmid was used.

Collaborators: Eddie Tabares (SB-2); Ivan Lozano (IP-4); Zaida Lentini (IP-4, SB-2)

3. Screening of the T0 + T1 NP plants to identify promising RHBV resistant transgenic rice plants.

- Six T1 transgenic rice lines were identified as having resistance to RHBV.
- At the T1 generation the trait for RHBV is still segregating.

The plants were not tested for resistance to RHBV until the T1 stage. Based on the inheritance analyses, the progeny of six selected T0 lines were evaluated for RHBV resistance. Approximately 100 progeny of each of the six T0 lines were inoculated at 35 days after planting by placing two proven vectors on each plant. The lines were rated for symptoms, disease severity, vigor, and yield to determine if the plants were resistant to RHBV. The controls were CICA 8 plants inoculated with two proven vectors or non-inoculated healthy CICA 8. After a 5 days period for the acquisition of the virus, the plants were sprayed and transplanted to larger pots. The plants were rated using a scale of 1 - 5. Plants showing no disease symptoms were rated 1 and those with mild symptoms limited to less than 10% of the leaf area were rated 2. Plants with typical symptoms covering 10-60% of the leaf area were rated 3, and stunted plants with extensive symptoms covering more than 60% of the area were rated 4. Plants that were highly necrotic or died were rated 5.

The disease rating in the T1 generation was highly variable. Many of the transgenic plants had extremely severe symptoms and were rated 4 or 5. Some lines showed some resistance with intermediate disease ratings. The most promising plants were progeny of the line A3-49. Many of the plants in this line were rated as two, and in some plants the symptoms were similar to local lesions. Six plants showing a high level of resistance were selected from this line including A3-49: 27, 37, 39, 56, 60 and 101.

Collaborators: Iván Lozano (IP4); Edwin Restrepo (IP4); Zaida Lentini (IP4, SB2);Lee Calvert (IP4)

4. Molecular analysis of the transgenic rice plants.

- The six lines of T1 RHBV resistant plants contained multiple copies of the N-gene plasmid construction.
- Four of the transgenic rice lines have at least one copy of the full length N-gene.
- Two of the transgenic rice lines contain truncated version of the N-gene.
- The N-protein could not be detected and RNA expression of the N-gene appears to be very low.
- It is thought that resistance is being conferred by the mechanism of gene silencing.

Southern blot analyses of the genomic DNA from the resistant T1 and T2 transgenic lines indicated that all the lines have multiple copies of the N-gene (Figure 3A) inserted in two sites. Plant DNA was also analyzed using set of primers to amplify the full length (primers sk/10), the 5'end (primers sk/2; sk/5 and Rev 3/5), and the 3'end (primers 10/8, and 10/6) of the N-gene (Figure 2). PCR analyses suggested that lines A3-49-27, -39, -56, and –60 contain at least one complete copy of the N transgene (Figure 3B). In contrast, lines A3-49-37 and –101 have truncated versions of the transgene showing the 5'end and 3'end regions of the genes but missing a region of about 500 bp corresponding to the central part of the gene (Figure 3C and 3D). These truncated N transgenes apparently sorted out as rearrangements through selfing. Interesting, these two lines having the truncated N transgene showed immunity (line A3-49-37) or the slowest rate of disease development (line A3-49-101, Figure 4), however line A3-49-37 showed very low fertility (Table 1).

While it is clear that the resistant lines contain the N-gene, the results with the expression of the RNA was not as consistent. Total RNA extraction followed by northern blot analysis did not detect N-gene transcripts in the plants. Reverse transcriptase PCR did detect RHBV N-gene RNA transcripts in some of the plants. Considering the very low level of the messenger RNA, it is not surprising that not N-protein could be detected in the RHBV resistant transgenic plants. Apparently there is no expression of the N-protein. Repeated attempts using highly sensitive methods have failed to detect the presence of the N.

Figure 2. A diagram of the DNA plasmid containing the RHBV N-gene and the approximate position of the oligonucleotide primers that were used in the PCR analysis of the RHBV resistant transgenic rice plants.

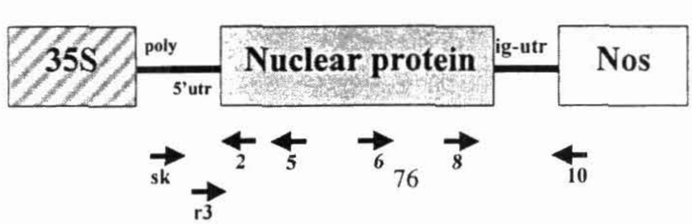
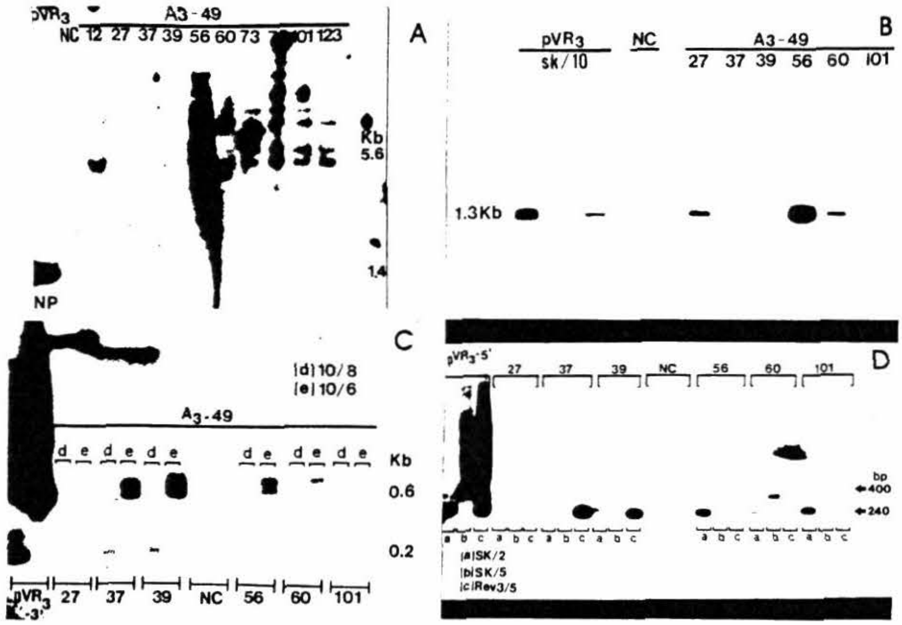


Figure 3. (A) Southern blot analysis of genomic DNA of Cica 8 transgenic plants carrying the RHBV nucleoprotein (N) gene. PCR analysis of the N gene corresponding to (B) full length; (C) 3'end; and (D) 5'end amplifications, respectively.



If there is only a low level of expression the N-gene messenger RNA and no expression of the N-protein, how is it possible that the plants are phenotypically resistant to RHBV? It is our current hypothesis that the mechanism of resistance is gene silencing. This mechanism is reported for tomato spotted wilt virus (ToSWV). ToSWV and RHBV are both members of the virus family bunyaviridae. Essentially, this mechanism of resistance operates by turning off the gene. Collaboratros: Iván Lozano (IP-4); Maritza Cuervo (ICW-83, IP-4); Edwin Restrepo (IP-4); Zaida Lentini (IP-4, SB-2); Lee Calvert (IP-4)

5. Assessment of the inheritance of the transgenes.

- Twenty six percentage of plants did not inherited the hygromycin resistance gene (ratio 0:1 resistant: susceptible) in the T1 progeny (Table 1).
- About 58% of the T0 lines showed a skewed segregation of 1:1 (resistant: suceptible), whereas 16% showed a segregation of 3:1 indicating the inheritance of a single active locus.
- The two lines that showed a 3:1 ratio for hygromycin resistance, also showed a 3:1 ratio for the presence of the hph and RHBV N genes in the T1 progeny, confirming the inheritance of a single active locus for the transgenes.
- However, those lines showing a 1:1 or 0:1 (resistant:susceptible) ratio for hygromycin resistance showed segregation ratio of 1:0 (homozygous) or 3:1 (heterozygous single locus) for the integration of the transgenes.

Nineteen T0 plants showing integration of the RHBV N-gene as indicated by Southern blots were analyzed for inheritance of the hph hygromycin resistance and RHBV N genes by genetic and molecular analyses of the transgenic T1 progeny. Genetic analyses were conducted by evaluating the resistance to hygromycin of T1 seeds germinated *in vitro*. Five of the nineteen (26.3%) plants did not inherited the hygromycin resistance gene (ratio 0:1 resistant: susceptible) in the T1 progeny (Table 1). About 58% of the T0 lines (10 of 19) showed a skewed segregation of 1:1 (resistant: susceptible), whereas 16% (2 of 19) showed a segregation of 3:1 indicating the inheritance of a single active locus (Table 1). Samples of nine plants including those with 1:1 or 3:1 segregation ratios were analyzed by Southern blots. The two lines that showed a 3:1 ratio for hygromycin resistance, also showed a 3:1 ratio for the presence of the hph and RHBV N genes in the T1 progeny, confirming the inheritance of a single active locus for the transgenes. However, those lines with a 1:1 or 0:1 (resistant: susceptible) ratio for hygromycin resistance showed segregations of 1:0 (homozygous) or 3:1 (heterozygous single locus) for the integration of the transgenes in the genome suggesting that the skewed segregations noted for hygromycin resistance are probably due to the inactivation of the hph gene (Table 1). Based on these results, five lines were selected (Table 1) for the RHBV resistance evaluations. The selection included lines with 1:0 or 3:1 segregation ratio for the N-gene according to the Southern blot analisis.

Table 1. Genetic and molecular analyses of the inheritance of the hph hygromycin resistance and RHBV N genes in the transgenic T1 progeny.

T0 line	Hygromycin resistance[1] observed ratio R:S	X^2	p	Southern blot[2] observed ratio Present:absent	X^2	p
A3-49*	1:1	0.34	0.56	1:0	------	------
A3-50	1:1	0.69	0.41	1:0	------	------
A3-57*	3:1	0.13	0.72	3:1	0.01	0.90
A3-58*	1:1	0.29	0.59	3:1	0.05	0.83
A3-59	0:1	------	------	NE		
A3-60	0:1	------	------	NE		
A3-61	0:1	------	------	NE		
A3-64	1:1	0.82	0.37	NE		
A3-72	0:1	------	------	NE		
A3-74	1:1	0.69	0.41	NE		
A3-75	0:1	------	------	3:1	0.00	1.00
A3-76	1:1	0.82	0.37	NE		
A3-77*	3:1	0.05	0.83	3:1	0.44	0.83
A3-78*	1:1	0.53	0.47	1:0	------	------
A3-81	1:1	0.09	0.76	NE		
A3-83	1:1	0.34	0.56	3:1	0.43	0.52
A3-84	1:1	0.29	0.59	NE		

[1] Twenty T1 seeds analyzed per T0 line. [2] Ten plants analyzed per T0 line, except for A3-57 where 23 plants were assayed, for the integration of the *hph* and RHBV-NP genes. * Lines chosen for RHBV resistance tests. NE= not evaluated.

Collaborators: Eddie Tabares (SB-2); Zaida Lentini (IP-4, SB-2)

6. Evaluation of the T2 trangenic plants for RHBV resistance.

- The trait for resistance to RHBV is still segregating at the T2 generation.
- Vigor and symptom severity are two important characteristics in determining the effectiveness of resistance to RHBV.
- T2 transgenic rice from the line A3-49-37 was nearly immune to the virus but has some abnormal characteristic.
- A3-49-56 and A3-49-101 were determined to be the lines with the most stable level of resistance to RHBV.
- The progeny for evaluation in the T3 generation were selected.

Self progeny from the six A3-49 lines were tested for the stability of the resistance. In a typical experiment 20 plants from each line were inoculated at 35 days after planting with two viruliferous vectors. After 5 days the plants were sprayed with insecticide and transplanted to larger pots. The disease rating scale was simplified to a three point scale. Plants with less than 10% of the leaves with symptoms were rated as 1. Plants with 10 to 60% of the leave are with symptoms were rated as 2. Stunted plants with more than 60% of the leaf area affected with symptoms were rated as 3. The intensity and severity of the symptoms were measured on individual leaves and then converted into an overall disease reaction rating for each plant. The presence RHBV was confirmed using an enzyme linked immunosorbent assay (ELISA). Whenever possible leaves showing symptoms were selected for the ELISA.

The line A3-49-37 was an exception line in that the agronomic characteristics were not normal and few seeds were collected. Nevertheless, four T2 progeny were tested because the T1 parent had almost immune response to RHBV. The T2 plants were nearly similar to the parent in having a very high level of RHBV resistance but abnormal agronomic characteristics including poor seed yield.

- To determine which lines were showing the most stable level of resistance, a comparison of vigor versus symptom expression was made (Table 2). A simple sorting classified the individual plants into nine categories. The best rating was a symptom rating of one with vigor of three. Sixty-one percent of the progeny of the line A3-49-56 were in this category, and 78% were rated as highly resistant although 17% only had vigor 2. Six percent of the progeny were highly susceptible and had poor vigor. This shows that the resistance trait is still segregating albeit most plants showed some resistance. This was rated as one of the best lines. Another promising line is A3-49-39. Even though only 33% were in the best category, 72% of the plants were rated with the highest level of vigor. This is despite 39% showing moderate symptom development. A3-49-60 was a highly variable line with 41% of the plants having poor vigor and only 47% having normal vigor. This same line had 31% with severe symptoms and 47% with mild symptoms. The distribution of the plants is one measure of the resistance being fixed in the line.

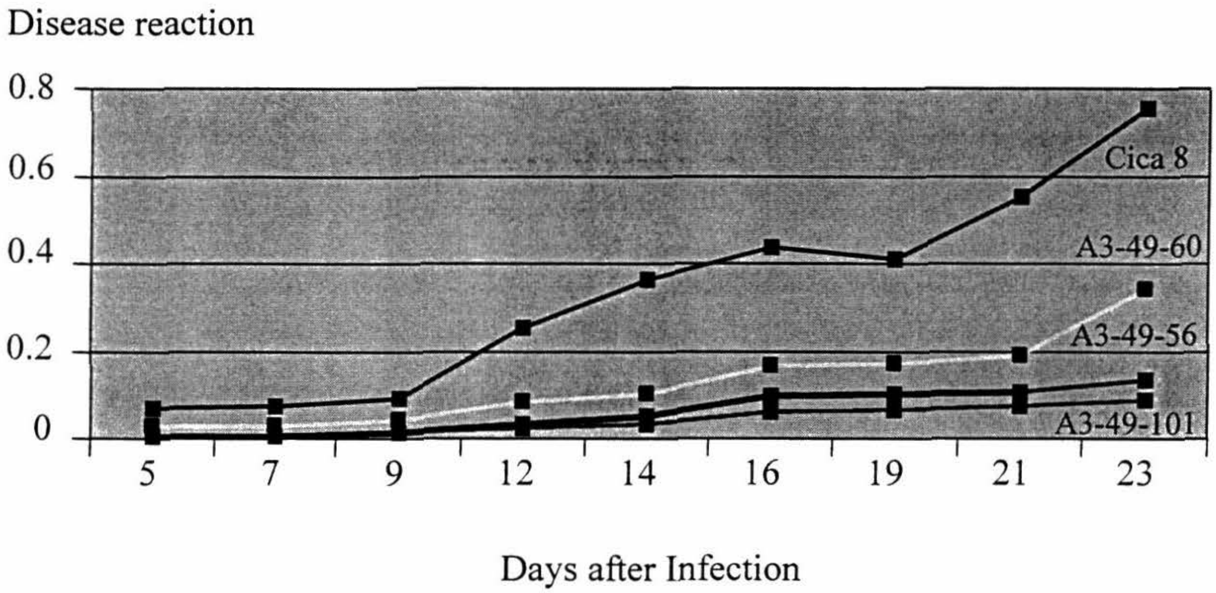
Table 2. A comparison of the vigor and symptoms of five transgenic rice lines inoculated with RHBV.

Linage	A3-49-27			A3-49-39			A3-49-56			A3-49-60			A3-49-101		
	Vigor Rating[1]														
Symptom Rating[2]	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
3	7	20	33	0	6	0	6	6	0	5	16	5	17	6	17
2	0	0	13	0	11	39	0	11	0	21	5	0	0	11	0
1	0	7	20	0	11	33	0	17	61	5	0	42	0	22	28

1. The vigor of the plants was based on number of tillers and plant height. Stunted plants with few tillers were rated 1 and plants with vigor similar to non-inoculated controls were rated 3.
2. The symptom rating is based on the intensity and severity of the symptoms. Less than 10% of the leaf area is a rating of 1, 10-60% a rating of 2 and more than 60% of the area with symptoms is a rating of 3.

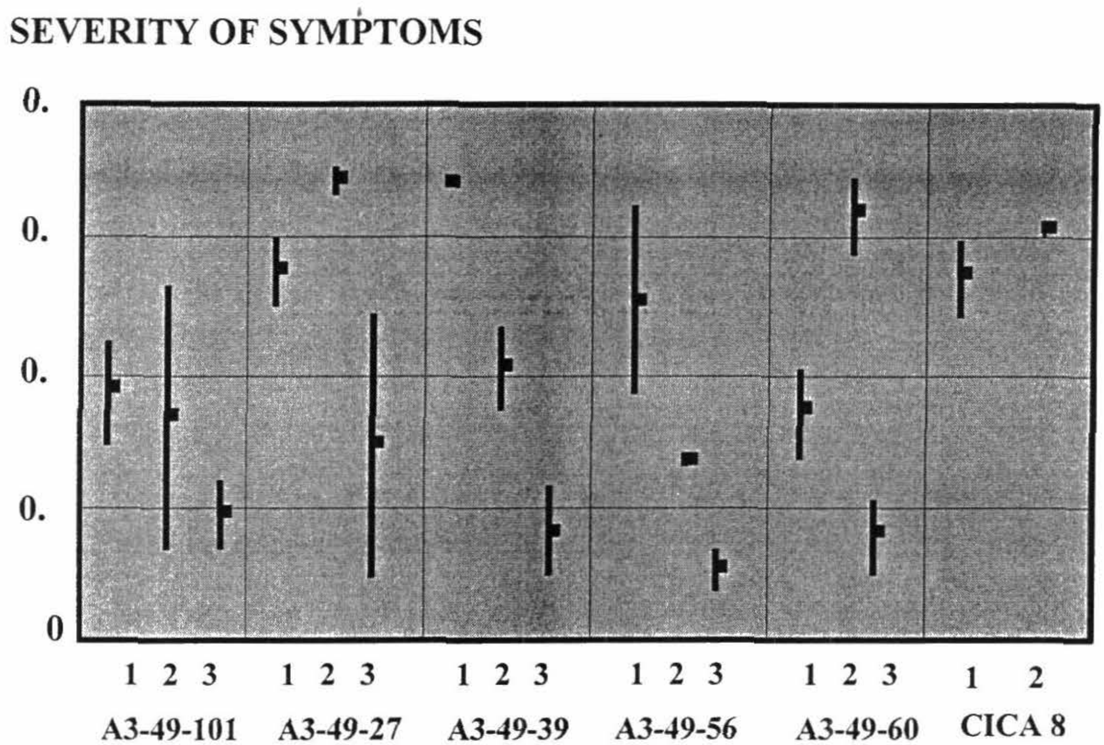
Another measure of resistance was the comparison of the disease reaction of the different group of plants. This was a measure of the symptom intensity. A disease index rating was calculated from detailed analysis of the symptom development. Every individual leaf of the plants was counted and rated for the severity of the virus lesions every two days. The disease reaction of each plant was made by calculating the number of leaves affected, the average of the symptom development per leaf and the vigor rating of the plant. All plants in the group were then average together. A comparison of three transgenic lines with the control of CICA 8 is shown (Figure 4). At 12 days after the period of virus acquisition, the different disease reactions become apparent. There was not a delay in the onset of symptoms, but the symptom developments was much less in the plants consider to be RHBV resistant. By 23 days, the differences in the disease reaction were distinct. CICA 8 is a susceptible variety and it had the highest disease reaction. The line A3-49-60 had an intermediate disease reaction rating, and A3-49-56 and A3-49-101 had the lowest disease reaction rating. This correlates well with the results made by sorting the plants according to vigor and symptoms giving a good degree of confidence that we are indeed selecting rice with resistance to RHBV.

Figure 4. RHBV disease development in Cica 8 non-transgenic plants and transgenic lines A3-49-56, -60, and –101. The points represent the averages of approximately 20 plants. Disease reaction refers to the combination the number of leaves with symptoms per plant and the severity of the symptoms. A disease reaction of 1.0 (maximum rating) indicates that all the leaves of the plant were fully covered by RHBV symptoms.



Since the population is still segregating for resistance to RHBV, the degree of the stability of the resistance trait is still of concern. Because we are analyzing resistance and not immunity, traits other than successful infection were needed to sort the plants within a line. Vigor was found to be an effective trait for explaining a large part of the variation. After ranking the plants in each line by vigor, a statistical analysis of various traits were made. A comparison of symptom severity in the three classes of vigor for five lines showed that there was a correlation with the highest vigor and low symptom severity (Figure 5). The surprising result was that plants with intermediate vigor often had more severe symptoms than those with the lowest level of vigor (i.e. A3-49-27 and A3-49-57). This emphasizes the importance of using multiple criteria in the selection of the plants for the subsequent generation. The best plants from each line were selected by using the criteria of highest vigor and low disease severity. After producing a ranking using those criteria, the plants with the highest yield were the plants chosen for analysis of the T3 progeny.

Figure 5. A comparison of severity of symptoms after grouping the plants according to vigor.



Collaborators: Ivan Lozano (IP-4); Edwin Restrepo (IP-4); Zaida Lentini (IP-4, SB-2); Lee Calvert (IP-4)

7&8. Evaluation of the T1 + T2 transgenic RHBV resistant plants for agronomic traits and assessment of its stability throughout generations

- The eight *T1 lines* derived from the A3-49 T0 line showing attenuated disease symptoms also showed an increased performance for various agronomic traits respect to the non-transgenic control infected with RHBV.
- Some of the infected T2 transgenic plants from lines A3-49-56 and A3-49-101, which have the most stable level of resistance, showed a yield similar to the non-infected non-transgenic control Cica 8 suggesting that the N-gene conferred a complete tolerance to those plants.
- A3-40-56 showed to be the best line showing a stable resistance from T1 to T2 generation jointly with good agronomic traits and fertility, and high yielding potential.

The 8 T1 lines derived from the A3-49-T0 line showing attenuated disease symptoms (*Section* 6), also showed an increased performance for various agronomic traits respect to the non-transgenic control infected with RHBV (Table 3).

Table 3. Evaluations of T1 progeny plants from line A3-49 for RHBV resistance and agronomic traits under greenhouse conditions.

Line A3-49		Flowering (days)	Height (cm)	Tillers	Flowers/ panicle	Grains/ plant	Fertility (%)	Disease reaction	ELISA (units)
27		121	55	8	67	110	23	2	0.568
34		127	55	44	54	5	9	3	0.153
37		124	62	23	56	40	3	1	0.071
39		126	57	15	63	287	22	2	0.372
56		126	86	12	83	696	70	2	0.558
60		133	66	12	114	396	32	2	0.787
75		125	78	15	67	147	16	3	0.152
101		113	78	14	71	376	28	2	0.432
Cica 8									
Infected	mean	141	66	5	53	0	0	4	0.440
	Sd	29	15	4	55	0	0	1	0.111
Not	mean	117	83	15	106	1594	87	1	0.005
infected	Sd	11	3	8	18	454	8	0	0.001

1 (dead plant); 2 (diseased, low vigor); 3 (diseased, intermediate vigor); 4 (vigor, some tillers free of symptoms); 5 (healthy looking plant).

The T1 lines A3-49: 27, 37, 39, 56, 60, and 101 selected for its level of RHBV resistance were selfed and the progeny seeds (T2 generation) of each of these T1 lines was evaluated for its agronomic performance. The increase level of resistance of the transgenic lines was also reflected in the significant increase of the yield potential (grains/plant) respect to the infected non-transgenic Cica 8 (Table 3). Some of the infected T2 transgenic plants from lines A3-49-56 and A3-49-101 showed a yield similar to the non-infected non-transgenic control Cica 8 (Table 3, maximum value) suggesting that the N-gene confers a complete protection to those plant. Of the agronomic traits evaluated the numbers of tillers showed a consistent significant increased in the T2 transgenic lines respect to the infected non-transgenic control, and no difference was noted respect to the non-infected non-transgenic control (Table 3). These results indicate that the N-gene protects the plants from the very early stage of development allowing a normal vegetative growth of the plants.

Table 4.- Yield and tiller potential of T2 progeny plants from Cica 8 derived transgenic lines.

Line A3-49		Grains/ plant Mean	SE	Min	Max	Tillers Mean	SE	*Minimum*	*Maximum*
56		448*	74	100	1332	15*	1.9	5	19
60		302	46	81	728	14*	1.1	5	20
101		364	73	79	1240	15*	1.6	5	30
Cica 8									
Infected	mean	70	28	26	122	5	1.3	1	7
	Sd	48				3			
Not	mean	1218*	198	825	1465	13*	0.6	12	14
infected	Sd	343				1			

Significant different at p: 0.01. SE = standard error. Sd = standard deviation.

Collaborators: Eddie Tabares (SB-2); Iván Lozano (IP-4); Edwin Restrepo (IP-4); Zaida Lentini (IP-4, SB-2); Lee Calvert (IP-4)

9. Preparation of seed sources for field testing.

- Seeds of resistant T2 lines (T3 progeny) from A3-49-27, 34, 37, 56, 60, and 101 is being multiplied under greenhouse conditions.
- The T3 progeny from these lines will be used for field testing when permit for Costa Rica (in process) and Colombia (when biosafety regulations approved) is acquired.

With the aim of testing the performance for RHBV resistance and agronomic traits, seeds of various resistant T2 lines (T3 progeny) from A3-49-27, 34, 37, 56, 60, and 101 is being multiplied under greenhouse conditions. Panicles of each transgenic plant are bagged to avoid possible pollen contamination from neighbor plants. Selected material includes the most resistant lines and the best performance for agronomic traits to conduct field trials by the first semester of 1998. Initial contacts have been made with the Costa Rican local authorities, since Costa Rica has approved biosafety regulations for field testing and Costa Rica will be one of the beneficiaries of this technology. Besides Costa Rica, another choice for initial testing will be Colombia, once the biosafety regulations are approved at the beginning of next year.

Collaborators: Eddie Tabares (SB-2); Zaida Lentini (IP-4, SB-2)

10. Crossing transgenic RHBV resistant plants with other desirable parents for use in conventional breeding programs

- The best lines from A3-49-56, 60 and 101 will be crossed with chosen genotypes to study the N gene expression, and tranfer the N-gene into selected breeding materials.
- The F1 crosses will be evaluated for stability of the N resistance into susceptible varieties, and for the complement of the N resistance gene with the genetic resistance for RHBV present in commercial varieties.

Selfed seeds from the best lines for RHBV resistance and agronomic traits will be crossed with the breeding line CT8008 (susceptible), and the varieties Oryzica 1 (intermediate resistant), FB007 (resistant), and Cica 8 (susceptible, control). Fifty F1 seeds of each cross will be assayed for the presence of the N gene by PCR, and 24 F1 plants containing the gene will be evaluated for RHBV resistance under greenhouse conditions. This study will allow to determine the expression of the N resistance on different genotype background, and if the N-gene can be used as a complement of the genetic resistance for RHBV available in some commercial varieties, for protecting the plants at ages younger than 25 day old.

Collaborators: Carlos Gamboa DANAC-Venezuela (M.Sc. Thesis); James Gibbons (FLAR); Iván Lozano (IP-4); Luisa Fernanda Fory (IP-4); Lee Calvert (IP-4); Eddie Tabares (SB-2); Zaida Lentini (IP-4, SB-2)

11. Acquiring permission for field testing of transgenic plants.

- T3 transgenic rice with resistant is ready to begin field testing

Now that there are sufficient seed of the T3 generation, field testing of the material for resistance needed. The Colombian government is in the process of developing the guideline for the testing and deployment of transgenic plants and no field testing will be done at CIAT until those regulations are in place. We expect the regulations to be ratified during the next few months.

The possibility of field testing in Costa Rica in collaboration with Dr. Ana Mercedes Espinoza was considered. Costa Rica has the guidelines in place and their biosafety officer assures us that it is easy to get permission for field testing in Costa Rica. To do the test in there be more expensive than at CIAT.

Transgenic crops are currently being grown on millions of hectares in the United States. The major transgenic crops are corn, soybean and cotton. A silenced RHBV N-gene in rice poses no risk either to the environment. Quite contrary successful deployment would lead to less use of toxic pesticides.

Collaborators: Zaida Lentini (IP-4, SB-2); Lee Calvert (IP-4)

12&13. Transform rice with RHBV NS4 nonstructural protein antisense contructs.

- A total of 165 Cica 8 plants from the antisense RHBV-NS4 were obtained.
- Southern blot of genomic DNA and Northern blot of the plants recovered from the antisense RHBV-NS4 bombardments indicated that 2 of these plants (1.2 %) contain and express the antisense-RNA4 gene.
- The NS4 gene was not inherited into any of the T1 generation of these two T0 plants, which it did not allow to proceed with the RHBV evaluations.

Construct containing the antisense RHBV-NS4 genes driven by the 35S CaMV promoter was used. The protocol used in this case to generate and recover the transgenic plants was the same as for the NP gene described above. A total of 165 Cica 8 plants from the antisense RHBV-NS4 were obtained. Southern blot of genomic DNA and Northern blot of the plants recovered from the antisense RHBV-NS4 bombardments indicated that 2 of these plants (1.2 %) contain and express the antisense-RNA4 gene. However, the NS4 gene was not inherited into any of the T1 generation of these two T0 plants, which it did not allow to proceed with the RHBV evaluations. The generation of transgenic plants carrying other versions of the RHBV-NS4 and RHBV-NP sense and antisense to modulate different levels of the RHBV transgene expression is also in progress. Future work will include the genetic and molecular characterization of these plants jointly with the RHBV resistance evaluations to determine the efficiency of the different strategies to confer protection to this virus.

Collaboratos: Eddie Tabares (SB-2); Ivan Lozano IP-4; Zaida Lentini (IP-4, SB-2); Lee Calvert (IP-4)

14. Preparation of article on transgenic resistant RHBV plants.

- A draft of the first article of two, describing the generation and performance for RHBV resistance and agronomic traits of Cica 8 transgenic plants has being prepared and it is under revision, to be submitted to Molecular Breeding by December 1997.
- A last set of molecular analysis is being conducted to complete the information needed for this first article.

Collaborators: Zaida Lentini (IP-4, SB-2); Lee Calvert (IP-4)

15. Development and training of staff.

- Carlos Gamboa, M.Sc. thesis, DANAC, San Felipe, Venezuela.
- Edwin Restrepo, Colciencias Scholarship & training course, Bogotá, Colombia.
- Mauricio Rodriguez, Laboratorio de Biotecnología, CORPOICA, Bogotá, Colombia.
- Hernando Ramírez, Ph.D. thesis, Universidad Nacional de Colombia, Palmira, Colombia.
- Leonardo Galindo, B.Sc. thesis, Universidad de Colombia, Palmira, Colombia.
- Ivan Lozano, M.Sc. thesis, U. de Valle, Cali, Colombia.

Expertise gained on transforming rice using biolistic system has been extended by training National staff for its use on rice, *Brachiaria*, sugar cane, and tomato, for introducing different traits of interest such as virus resistance, insect resistance, and disease resistance. Of the trainees listed above, the thesis students and the Colciencias scholar completed at least a year of work at CIAT, allowing them also to get acquainted with the molecular work associated with genetic transformation of plants.

Collaborators: Ivan Lozano (IP-4); Eddie Tabares (SB-2); Lee Calvert (IP-4); Zaida Lentini (IP-4, SB2)

OUTPUT 3. RICE PESTS AND GENETICS OF RESISTANCE CHARACTERIZED

C. MITIGATE LOSSES DUE TO RICE HOJA BLANCA VIRUS (RHBV) AND *Tagosodes orizicolus*

This is an integrated pest management project with a range of activities. The activities to enhanced germplasm pools with resistance against RHBV and *T. orizicolus* are essential and will continue indefinitely. The Colombian risk assessment survey started two years ago in response to increasing level of RHBV. This activity needs to be expanded to other countries in the region and should continue until the incidence of RHBV is declining. Epidemiological studies and virus/vector interactions are needed to develop a IPM program to control RHBV. These activities are expanding and in regards to control of the vector, other insects that cause economic damage and influence the use of agrochemicals can not be ignored. The development of IPM is an essential component to maximizing yield and reducing the cost of production. The other sub-project is the production of transgenic rice with resistance to RHBV. This is a novel source of RHBV resistance and will be incorporated into breeding programs. For transgenic plants to be most useful additional traits such as resistance to insect pests need to be incorporated into future activities.

1. Evaluate rice germplasm for resistance to *T. orizicolus*

Developed improved screening method to select for resistance to planthoppers.

Evaluations to incorporate diverse sources of resistance to *T. orizicolus* into gene pools and breeding lines are conducted throughout the year in greenhouse conditions. From the experience, with Llanos 5 a variety with a high level of resistance to RHBV but susceptible to the planthopper vector, the importance of combining both types of resistance is recognized.

To better screen germplasm for resistance to *T. orizicolus*, Andrés Ginarte (IIA, Cuba) worked with the entomology section to compare the evaluation methods use at IIA with those used at CIAT. Modifications were made in both methods and an improved system of evaluation of the mechanical damage caused by *T. orizicolus* was developed. This planthopper resistance screening method has been adopted by IIA, FLAR, FEDEARROZ and CIAT.

Table 1. Unified method for evaluation of mechanical damage caused by *T. orizicolus*.

Plant number	10 plants per rep (3 reps)
Inoculation date	15 days after planting (12 days after germination)
Number of insects per plant	10 or more adults per plant
Susceptible check	IR 8
Resistant check	Makalioka
Time of evaluation	When 80% of IR 8 plants are dead (approximately 25 days after inoculation)

During 1997, a total of 2911 lines evaluated and 54% were classified as highly resistant. The total number lines evaluated is 50% more than in 1996. Also the percentage of lines classified as resistant increased from 36% to 54%.

Collaborators: James Gibbons (FLAR); Mónica Triana (IP-4); Andrés Ginarte (IIA, Cuba).

2. Evaluate rice germplasm for resistance to RHBV

This year there was an increase in the percentage of rice lines that are resistant to RHBV indicating better incorporation of this trait in the rice gene pools.

Evaluations to incorporate sources of resistance to RHBV are conducted twice a year in the field. Trials from the second semester of 96 and the first semester of 1997 are included in this report. During this period, a total of 3158 line were screened. The lines that were evaluated were from CIAT, FLAR, FEDEARROZ and Colombia-CORPOICA, INGER, Costa Rica, Venezuela-NPAM, Surinam, Cuba-IIA, VIARC, and USA-LSU. A total of 1093 lines were evaluated in the field during the second semester of 1996, and 44.0% were classified resistant to RHBV. In the first semester of 1997, 2065 lines were evaluated and 44.7% were resistant. Compared to the previous year, there was an increase in the percentage of lines with RHBV resistance. During 1995 second and 96 first semester evaluations, only 27% of the lines screened were resistant to RHBV. For both traits, RHBV resistance and planthopper resistance, there is a large increase in the number of lines that have these traits. The increase in percentage of lines with a high level of resistance to RHBV and the planthopper vector is encouraging and reflects greater concern to incorporate these traits into commercial varieties.

Collaborators: Mónica Triana (IP-4); Ana Cecilia Velasco (IP-4); James Gibbons (FLAR); Lee Calvert (IP-4).

Activities 1 & 2

A new variety with a high level of tolerance to *T. orizicolus* and RHBV is about to be released in Colombia.

FB0007: New promising rice experimental line with resistance to Hoja Blanca Virus disease complex.

The experimental line FB0007 (FB0007-3-1-6-1-M) was selected from a group of 22 lines from FEDEARROZ (Colombian Rice Federation) which were being multiplied by FLAR (Fund for Latin American Irrigated Rice) for rapid inclusion in Colombian regional trials. It was selected due to its high level of resistance to the RHBV and the insect vector, *T. orizicolus*. FB0007 derives from the cross Llanos 4/5685 made in 1992 at Bosconia in the North Coast of Colombia. It is a late season variety with moderately erect plant type, large panicles and dark green foliage. It yields well in comparison to Colombian check cultivars and has acceptable long grain appearance and cooking qualities. In extensive tests in the major rice growing areas of Colombia it has shown milling quality results similar to CICA 8, the cultivar which it should replace.

Under high disease pressure at the Santa Rosa plant breeding site it has shown a moderate level of resistance to blast disease, and acceptable levels of tolerance to leaf scald and grain discoloration. Breeder and Foundation seed of this line are being produced at CIAT headquarters under the supervision of FEDEARROZ breeders and will be available to commercial seed producers at the end of 1997 or early 1998.

3. **RHBV colony maintenance**

The capacity of insect rearing facility was augmented.

This is a routine but importance activity for the rice virology and entomology. During 1997, the capacity for augmenting viruliferous colonies was significantly increased. There was an increase in the amount of germplasm that is being screened for resistance to RHBV and to *T. orizicolus*. Also there is demand for these services for the epidemiological experiments that are being conducted. During 1997, the insect rearing facility was renovated and additional space was found in a separate greenhouse. The separate facility increases capacity and acts as a reserve in case of problems with the main colony.

Collaborators: Mónica Triana (IP-4); Maribel Cruz (IP-4); Lee Calvert (IP-4); Fernando Correa (IP-4)

4. **Transfer of *Tagosodes* and RHBV evaluation technics**

CORPOICA has started a colony of *T. orizicolus*.

Epidemics are cyclic and outbreaks are regional. Therefore, one cannot depend on natural disease pressure to select germplasm with resistance to RHBV. Also, the virus is not readily mechanically transmitted. The only reliable method to select rice germplasm with RHBV resistance is to inoculate plants using highly viruliferous colonies of *T. orizicolus*. The planthopper can be genetically resistant to RHBV, and to maintain a viruliferous colony, one must continually select for susceptible planthoppers. Controlled crosses are made and the progeny are tested with serological methods (ELISA) to determine the percentage of vectors in the colony. Because it is costly to maintain, CIAT is currently the only facility using viruliferous colonies to screen for RHBV germplasm. COPROICA is starting a vector colony of *T. orizicolus*. This will increase the capacity to screen germplasm and permit multilocational testing. This should help assure the selection of durable resistance. A colony of the planthopper was started at the CORPOICA experiment station, La Libertad. The ELISA methods for vector testing have been transferred. The colony needs to be increased in size and CORPOICA should be ready to screen germplasm during 1998.

Collaborators: Judith Guevara (CORPOICA); Vicente Rey (CORPOICA); Mónica Triana (IP-4); Lee Calvert (IP-4)

5. Surveys of RHBV incidence in rice and *T. orizicolus*

The general level of RHBV is continuing to increase but at a steady not epidemic rate.

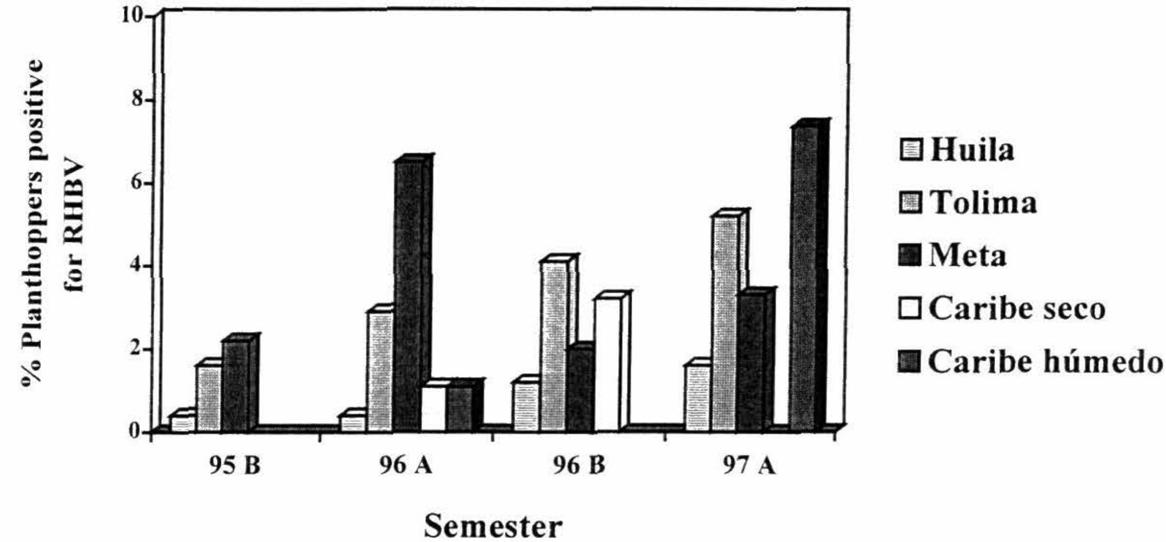
The Caribe humid zone is now considered to be a high risk for outbreaks of RHBV

In general the levels of planthoppers are being controlled by using insecticides. IPM is needed to reduce the risk that the planthoppers will become *insecticide resistant.*

This is the second year of a survey in the rice growing regions of Colombia to determine the risk of outbreaks of RHBV. Rice fields are selected and the population of *T. orizicolus* is estimated. Then samples of 100-200 *T. orizicolus* are collected and analyzed by serological methods for the presence of RHBV. Insects that were positive for the presence of the virus were considered to be vectors. The incidence of RHBV infected plants was collected from the same fields 45-60 days after the planting date. During the first semester of 1997, 75 samples of *T. oricolus* were analyzed to determine the percentage of viruliferous insects. This year there was an increase in the level of vectors in the humid Caribe zone. The percentage averaged 7.4% vectors, and there were some pockets with up to 20% vectors that were experiencing outbreaks of RHBV. There are still not enough samples from that region to make determine how widespread are the high levels of vectors. The central region including the departments of Tolima and Huila continue to have an steady increase in the percentage of vectors (Figure 1). Huila has gone from less than 0.5% in second semester 95 to 2% vectors in the first semester of 97. This is still a very low level and no control recommendations are being made. Tolima has increased from 2% in the first semester of 95 to 5.5% vectors in the second semester of 97. There is extensive information from these regions and RHBV is prevalent throughout Tolima especially in the northern region. It is recommended to grow resistant varieties and to control the populations of the planthopper. Even the llanos region is experiencing increasing levels of vectors. This is despite very heavy rains and less apparent total RHBV in the field. Heavy rains normally help depress the total population and is the probable reason for a generally lower level of RHBV than occurred in 1996. Nevertheless there is the potential for serious problems with RHBV in the llanos.

Besides the extensive monitoring that is being done throughout Colombia, information is being collected from other countries albeit in a less systematic manner. The worst reported outbreak of RHBV was in the late 1996 in Peru. In one region, very heavy losses (70-80%) over 30,000 hectares were reported. Both the Dominican Republic and Costa Rica have reported outbreaks of RHBV. The situation in Costa Rica is under control apparently by the very heavy use of pesticides. There are already reports of insecticide resistance and the need to spray mixes to control the planthopper. This highlights the need to develop and implement IPM. The overuse of insecticides can lead to highly resistant insects and the absence of natural enemies. If this happens, there could be extensive losses throughout Costa Rica.

Figure 1: Survey of RHBV viruliferous planthopper in five rice growing zones in Colombia



Collaborators: Luis Reyes (FEDEARROZ); Ana Cecilia Velasco (IP-4); Catherine Pardey (Colciencias); Lee Calvert (IP-4).

The Colombian survey relies on many collaborators in FEDEARROZ and CORPOICA.

Luis Castilla	FEDEARROZ	Alvaro Salive	FEDEARROZ
Martha Sánchez	CORPOICA	Educardo Arévalo	FEDEARROZ
Felix Hernández	FEDEARROZ	Alberto Dávalos	FEDEARROZ
Olga Higuera	FEDEARROZ	Luis Dussán	FEDEARROZ
Vicente Rey	CORPOICA	Edison Ortega	FEDEARROZ
Cristo Perez	FEDEARROZ	José Medina	FEDEARROZ
José Vargas	FEDEARROZ		

6. Vector / RHBV interactions

The genetics of planthopper resistance to RHBV is more complicated than previously believed.

One objective of studying vector/virus interactions is to facilitate making more stable colonies that remain in highly viruliferous with less active management. Another objective is develop early warning system for RHBV risk assessment by being able to rapidly determine the genetic frequency of RHBV resistance gene in planthopper populations. Molecular methods that replace the current biological assay would allow this type of analysis to be done over the entire region.

T. orizicolus is a host of RHBV, and the virus replicates within the insect. It is reported that the ability to acquire and support replication of RHBV is mediated by a single recessive gene. This R gene is an insect gene for resistance to RHBV that is reported to be incompletely dominant. In some crosses the dominance is not complete and there is a low frequency of vectors from crosses of nonvectors. The other phenomena that must be considered is transovarian transmission. The virus passes at a very high frequency from mother to the progeny. Some of the nymphs that contain the dominate R gene still are able to transmit the virus. It is suspected that the masking effect of transovarian transmission makes it difficult to develop colonies the are 100% homozygous recessive (rr) for the R gene.

Crosses of male vectors and female nonvectors were made. This eliminates any transovarian effects and was done to generate the populations needed to develop molecular markers. The F1 progeny were tested for the ability to transmit virus using a plant assay. There were two types of reactions. In some of the crosses, there were no vectors in the F1 population. In the other group, there was an average of about 14% vectors. Based on a single recessive gene, it is predicted that these crosses should produce either no vectors or 50% vectors. The highest level of vectors in any of the F1 crosses was 18.2%. The biological test only measured the transmission not the ELISA+ planthoppers. We chose the non-destructive test because the progeny were needed to make the F1 crosses. This result is apparently different from the single resistance gene that has been reported. To further confuse the issue, in most of the F1 crosses, only a small percentage (less than 4%) of the progeny could vector RHBV. The results were not as expected but the experiment was to generate progeny to look for a molecular marker not test the hypothesis that a single recessive gene conferred RHBV susceptibility to the planthopper. A set of controlled crosses is being made to better understand the genetic of RHBV in the planthopper.

A vector and non-vector from the F1 were crossed and 37.7% of the progeny were able to transmit RHBV. The vectors and non-vectors from this cross were bulked together and DNA was extracted. Then a total of 90 ten base oligonucleotide (Operon, Inc.) were tested to identify a molecular marker to either resistance or susceptibility. The results were not reproducible and no marker was identified. The technique of amplified fragment length polymorphisms (AFLP) was done and there are several promising candidate bands. Further research is need to determine if any other AFLP products are indeed true markers.

It is clear that we need to better understand the genetics of the planthopper resistance to RHBV. There always have been stability problems in the viruliferous colony and the maternal transmission masking the resistance gene was considered a principal reason for this instability. The current evidence suggests that the genetics of resistance is more complex than previously thought. Before we can expect to understand the dynamics of the vector population in the field, we need a clear concept of the genetics of resistance.

Collaborators: Iván Lozano (IP-4); Lee Calvert (IP-4).

7. RHBV epidemiological studies

The development of recommendations for varieties to grow in areas with a high risk of RHBV outbreaks.

A two field trials were made to determine the response of varieties to different levels of disease pressure. The results of the two trials were very similar but the evaluation method for disease pressure was simplified in the second trial. In the trials, 6 commercial varieties and two advanced lines were tested for field tolerance. There were three levels of disease pressure including the release of ca. 3 insects per plant, 1.5 insect per plant and a control without any release of insects. The virulence of the planthopper colony at the time of release was ca. 80%. The insects were released at 15 days after planting. The results were that the CT 8222, FB0007, O. Llanos 5 and Oryzica 3 had no significant loss of yield at either level of disease pressure. Oryzica 3 was somewhat different from the other three because it had a higher disease rating but the yield was not affected. This raises the question of the mechanisms of resistance. How much of the resistance is from the exclusion of the virus and what is the effect of tolerance to the virus. This will be addressed in a series of experiments that will rate the tolerance of infected plants of each variety. Although the variety Oryzica 1 has field resistance, in these trials it had significant yield losses. In the experiment reported in the Table 1, the disease rating of Oryzica 3 and Oryzica 1 was similar at the low disease pressure but at the higher level Oryzica 1 proved to be more susceptible. At both levels the yield losses were significant less as compared to the control treatment.

The experiments confirms that the two advanced rice lines have high levels of field resistance. The commercial varieties, O. Llanos 5 and Oryzica 3 proved to be the best commercial varieties that were tested. Both of these varieties are not widely accepted and it is hoped that after it is introduced, FB0007 will become a popular variety. The yield of Oryzica 1 was disappointing in these trials. It is still recommended as having intermediate level of resistance but under heavy RHBV pressure it may sustain economically important losses. These experiments help develop the variety recommendations for those zones that are identified at risk of outbreaks of RHBV. Additional varieties are being incorporated into the current trials.

Table 2. A comparison of the effect of different disease pressure on the incidence of RHBV and the effect on the yield on six commercial varieties and two advance rice lines.

Rice variety	Inoculation level					
	High		Low		No Inoculation	
	RHBV[2] Incidence	Yield (kg/ha)	RHBV incidence	Yield (kg/ha)	RHBV Incidence	Yield (kg/ha)
CT 8222	1.24 f	5519[3] abc	1.00 fg	5585 abc	0.03 h	6377 abc
FB 0007	1.62 f	6378 abc	1.23 f	6963 ab	0.07 h	6374 abc
O. LLANOS 5	1.69 f	5518 abc	1.40 f	6408 abc	0.05 h	6118 abc
ORYZICA 3	2.91de	6352 abc	2.50 e	6441 abc	0.07 h	7005 a
ORYZICA 1	4.60 c	2799 de	3.32 d	3491 d	0.12 h	5098 c
O. CARIBE 8	8.40 a	2957 de	7.28 b	2032 fe	0.23 h	5401 bc
CICA 8	8.60 a	1219 fg	8.56 a	510 g	0.35 hg	6496 abc

[1] High = ca. 3 insects/plant; Low = 1.5 insects/plant.
[2] Visual rating (0-9) 0= 0 %; 1= 1-10 %; 3= 11-30 %; 5= 31-50; 7= 51-70; 9= 71-100 % infected with RHBV.
[3] Average 6 replications. The letter represent a significance at P = 0.05.

Collaborators: Luis Reyes (FEDEARROZ); Maribel Cruz (IP-4); Lee Calvert (IP-4).

8. Greenhouse trials for the biocontrol of *Tagosodes orizicolus*

Entomopathogens to control *T. orizicolus* in greenhouse conditions were identified and are being tested in the field.

In collaboration with AgrEvo. S.A., a project to evaluate the effectiveness of isolates of entomopathogens for the control of *T. orizicolus* was started. The first part of the experiment consisted of screening isolates in the greenhouse. Forty-three isolates provided by AgrEvo were screened and mortality was the primary characteristic for selection of the best isolates. *Metarhizium anisopliae, M. flavoviride,* and *Bauveria bassiana* were tested. The isolates of *M. anisopliae* were the most efficacious with an overall mortality of 80.6%. This compared to 47.5% for *B. bassiana*, 24.2% for *M. flavoviride*, and 15.8% mortality in the control. These results were statistically significant at the 0.05 level. The affected insects especially the females lost mobility and fecundity. The mortality began at 6 days after inoculation, especially with *M. anisopliae*. The first instar nymphs were not affected by the isolates that were evaluated. The best isolates were selected and field trials were started to test the efficacy in agricultural conditions. The addition of entomopathogens in an IPM strategy is the objective of this activity.

Collaborators: Olga Lucía Higuera (FEDEARROZ).

9. Study on the genetic capacity of *T. orizicolus* to vector RHBV.

An early indicator that identifies hotspots before an outbreak of RHBV has been developed.

This is an activity that is part of epidemiology and virus/vector interactions. An article entitled "Determinación de vectores virulíferos de *Tagosodes orizicolus* al virus de la hoja blanca en zonas arroceras de Colombia" was reviewed by the CIAT publication committee and was submitted to SOCOLEN.

The experiment was designed to force the planthoppers to feed on RHBV infected plants and determine the percentage of insects in which the virus could replicate and how many could transmit the virus. This can only be done for a small number of samples and one or two field from each major region can be tested. The methodology consists of collecting nymphs from the field and transporting the live insects back to CIAT. The nymphs are placed on RHBV infected plants and then each planthopper is transferred to a healthy rice plant. The sample size was 180 individuals. The total number of insects with the genetic capacity to acquire the virus was determined by ELISA, and the ability to transmit RHBV by a plant assay. In the same field that nymphs were collected, a sample of adult planthopper were captured. These were assayed by ELISA and the positive insects were considered to be viruliferous vectors.

There was a very high correlation between the ability for RHBV to replicate in the planthopper and the ability to transmit the virus. In all cases the ability to acquire the virus was equal or just slightly higher than the ability to transmit the virus (table 3). This implies that the major form of planthopper resistance to RHBV is preventing the replication of the virus. The percentage of total vectors was always much higher than the viruliferous vectors collected from the field. This is expected until a peak of an epidemic. The highest level of infected plants was only 10% and this is evidently still not sufficient for all the insects with the genetic capability to become vectors.

Table 3. A comparison of the total number of *T. orizicolus* with the genetic capability to transmit RHBV, the number of viruliferous vectors and incidence of RHBV in the field.

State	Zone	Variety	Total % ELISA positive insects[1]	Total % Vectors Plant assay[2]	% Viru-liferous Vectors in field[3]	% of Plants RHBV in field[4]	Plant-hoppers (10 DP)
VALLE	Jamundi	Selectra 320	5.9 b[5]	4.7 b	1.1 c	0.7 d	4250
CESAR	Valledupar	O. Caribe 8	9.6 b	9.1 b	5.3 a	0.9 d	4
META	Castilla	Oryzica 1	21 a	19 a	9.3 a	6.3 c	22
TOLIMA	Saldaña	Oryzica 1	4.4 b	4.3 b	1.6 bc	0.3 d	8
TOLIMA	Ambalema	Oryzica 1	16.7 a	15.3 a	8.3 a	10.0 b	N/D

1- Insects were collected as nymph; fed on RHBV infected plants and assayed 25 days after the virus acquisition by ELISA.
2- The percentage was determined by visual symptoms on the plants.
3- The percentage of planthoppers in the field that were RHBV positive as determined by ELISA (180 insects were tested per locality).
4- The percentage of RHBV infected plants in the field was calculated by counting all the tillers in $0.5m^2$.
5- The letters are for significant difference at the P=0.05 level using chi-square analysis.
6- N.D. Data not collected.

There were some unexpected results such as the field in Valledupar in which over 5% of the planthoppers were vectors but the incidence of RHBV was less than 1%. This may be occur because a there had been a recent migration of planthopper to the field. This information is supplementing the Colombian RHBV survey. It confirms the survey data that the Ambalema region in Tolima and the Castilla region in Meta are hot spots for RHBV. The field at Jamundí in which only 0.7% of the plants were infected with RHBV and the field vectors were 1.1% demonstrates the predictive value of this type of testing. With the high populations of planthoppers and the potential for 5% of this population to transmit RHBV, the potential for a rapid increase in RHBV is possible. It also suggests that with a 9% level of total vectors, the Caribe seco regions around Valledupar are at higher risk than the survey has indicated. Understanding the relationship between the population of the planthopper, the total number of potential vectors, the number of viruliferous vectors and the incidence of RHBV in the field is a complex task that will allow early warning to farmers and is essential in designing more effective IPM strategies.

Collaboratos: Luis Reyes (FEDEARROZ); Ana Cecilia Velasco (IP-4); Lee Calvert (IP-4)

10. Coordination of COLCIENCIAS project to mitigate losses due to RHBV.

As project leader of the COLCIENCIAS grant "Control y epidemiología de virus del hoja blanca de arroz", I have coordinated the research activities are being conducted in CORPOICA, FEDEARROZ and CIAT. This grant is supplement the core activities of these three institutions and is a vital source of funding in the effort to break the cycle of RHBV epidemics.

Collaborators: Lee Calvert (IP-4); Miguel Diago (FEDEARROZ).

11. Development and training of staff

Ivan Lozano is studying for a M.Sc. degree at the U. of Valle. His thesis includes the development of transgenic rice resistant to RHBV and vector/virus interactions.

Catherine Pardey is a COLCEINCIAS becario who has been working activity of monitoring the virulence levels of *T. orizicolus*.

12. Training of personnel from NARs and NGOs

Judith Guevara of CORPOICA has received training in the methods of manipulating planthopper colonies. Special emphasis was given to methods to develop and maintain colonies with a high level of virulent planthoppers. The ELISA method was also part of this training.
Collaborator: Mónica Triana (IP-4)

Presentations on strategies to control RHBV have been made to groups of farmers, to agricultural technicians and other groups.
Collaborator: Luis Reyes (FEDEARROZ).

13. Popular article on RHBV

A short popular article about RHBV was published in the Revista Arroz. This is to disseminate information about the extent and nature of the current outbreaks of RHBV in Colombia. This article will be simplified and made into a brochure for distribution to rice producers.

La Hoja Blanca del Arroz. Revista Arroz V. 46 N. 409 pages 4-8.

Collaborators: Luis Reyes (FEDEARROZ); Octavio Vargas (FEDEARROZ); Lee Calvert (IP-4).

OUTPUT 3. RICE PESTS AND GENETICS OF RESISTANCE CHARACTERIZED

D. ISOLATION AND CHARACTERIZATION OF THE CAUSAL AGENT AND VECTOR OF "ENTORCHAMIENTO"

Executive Summary

In 1991, a new production problem known as "crinkling", emerged as a serious threat to rice production in the Eastern Plains (Meta) of Colombia. The problem was initially diagnosed as feeding damage caused by an aphid, and, later on, as nematode damage. These incorrect diagnoses resulted in the massive application of highly toxic systemic pesticides, which cost rice growers in the region over $ 4,000,000 USD in three years, but that did not control the problem. The etiology of the "crinkling" problem of rice was elucidated in 1994 at the Virology Research Unit of CIAT, as a new viral disease of rice in the Americas. This viral disease had been originally described from Ivory Coast, West Africa, in 1977.

The purpose of this project, is to characterize the causal agent and its fungus vector, in order to implement integrated disease control measures, and reduce environmental contamination due to the application of unnecessary pesticides currently used to control the "crinkling" disease of rice. Additionally, the project seeks to investigate the development of the disease in West Africa and in Latin America, to gain some knowledge on its pattern of dissemination and devise effective control methods.

The causal virus has been identified as **rice stripe necrosis** and its vector has been tentatively identified as a species of ***Polymyxa***. The causal agent belongs to the genus **Furovirus** and its main physical and chemical properties have already been characterized. Collaborative work between CIAT and WARDA has demonstrated that the virus causing the "crinkling" disease of rice in Colombia, is closely related to the rice stripe necrosis virus (RSNV) originally isolated in West Africa. Thanks to this project, the current distribution and economic importance of RSNV in West Africa is being investigated. So far, the "crinkling" disease of rice in Africa has been observed in Senegal, Guinea, Sierra Leone, Liberia, Ivory Coast, Ghana, Togo and Nigeria. In Colombia, RSNV has already been recorded in the main rice-growing regions of the country, namely: Cordoba, Antioquia, Tolima, Huila, Cundinamarca, Meta and Casanare.

Fortunately, the virus does not seem to be present in other Latin American countries, although similar symptoms have been observed in Nicaragua, Central America. In Argentina, a fungus similar to the vector of RSNV has been found affecting rice (causing foliar malformation) but the rice plants are not infected by RSNV. An informative leaflet has been distributed to Rice Programs in Latin America in order to halt the dissemination of the virus in contaminated rice germplasm or commercial seed.

Preliminary surveys conducted in isolated rice-growing regions in Colombia, demonstrate that over 45% of the farmers are already aware of the viral nature of the "crinkling" disease of rice, and that the volume of nematicides and insecticides has been reduced to less than half of the dosages applied a year ago.

Background

In 1991, several rice fields in the Eastern Plains of Colombia (Meta) presented an unusually high incidence of seedling death, and some of the surviving adult plants in those fields showed leaf striping, severe malformation and systemic necrosis. These symptoms became collectively known as the "entorchamiento ("crinkling") problem " by concerned rice growers in the region. By 1994, yield losses due to "entorchamiento" were estimated at 20%, and the problem appeared in other Departments (Arauca and Casanare) of the Eastern Plains, threatening rice production in over 80,000 hectares.

The Colombian Institute of Agricultural Research and the Colombian Rice Growers' Federation rapidly initiated research to determine the causal agent of the "entorchamiento" problem. The first "causal agent" proposed was the aphid *Rhopalosiphum rufiabdominalis,* causing an increase in the application of insecticides in the entire region. Unfortunately, the "entorchamiento" problem continued to expand despite the intensive use of pesticides to control aphids. A second investigation claimed that the causal agent was not the aphid but, rather, nematodes of the genus *Pratylenchus*. This report was soon in the hands of desperate rice farmers, who initiated the application of expensive and highly toxic (category I= highly hazardous) systemic nematicides to the soil and to irrigated fields using aircraft. Despite the intensive use of these highly toxic and broad spectrum pesticides, the "ëntorchamiento" problem of rice continued to spread in the region and in the main rice producing regions of Colombia.

The cost of pesticides and other control measures, such as increasing seed (plant) density, is estimated at four million dollars per year, and production costs/ha have increased $ 100 USD in affected rice fields. The health and environmental consequences of the use of nematicides lethal to small mammals, beneficial insects and fish, has not been determined yet.

I view of this situation, the Virology Research Unit of CIAT initiated research on this critical production problem, considered the second most important disease of rice in Colombia. By 1995, the causal agent of the "entorchamiento" disease of rice had been identified as a virus transmitted by a soil-inhabiting fungus of the genus *Polymyxa*. This virus had been previously (1984) identified in West Africa but the disease did not receive much attention following the departure of the European virologists, who diagnosed the problem in Ivory Coast.

Activities 1-5. Identification, isolation and characterization of the causal virus and its fungal vector.

The partial characterization of the causal agent of rice stripe necrosis has been completed, and a manuscript has been prepared to present the results of this investigation. The abstract of this manuscript is included below.

DETECTION OF RICE STRIPE NECROSIS VIRUS IN SOUTH AMERICA

F.J. Morales, Virologist; **J.A. Arroyave**, Electron Microscopist; **M. Castaño**, Research Associate; **G. Guzmán**, and **A.C. Velasco**, Research Assistants, Virology Research Unit, International Center for Tropical Agriculture (CIAT), Apartado Aéreo 6713, Cali, Colombia.
Corresponding author: F.J. Morales, E-mail: f.morales@cgnet.com

ABSTRACT

Morales, F.J., Arroyave, J.A., Castaño, M., Guzmán, G., and Velasco, A.C. 1997. Detection of rice stripe necrosis virus in South America.
A new disease of rice, known as "entorchamiento" (crinkling), was first noticed in the Eastern Plains of Colombia, in 1991. Symptoms include seedling death, foliar striping and severe plant malformation. Tissue extracts and partially purified preparations from diseased rice plants, contained virus-like particles ca. 20 nm in diameter, with a bimodal length of 260 and 360 nm. Particle aggregates were also observed in the cytoplasm of infected leaf cells. Electrophoretic analyses of purified preparations and ds-RNA extracts, revealed a single protein species of M_r *22,500, and four* ds-RNA bands ca. 6,300, 4,600, 2,700 and 1,800 bp in size. Cystosori, characteristic of plasmodiophorid fungal vectors of plant viruses, were consistently observed in the roots of diseased rice plants. The crinkling symptoms were reproduced by planting rice in soil collected from affected fields. The "entorchamiento" disease of rice in Colombia, is identical to "rice stripe necrosis" described in West Africa, in 1977. Rice stripe necrosis is caused by a furovirus (RSNV) transmitted by the fungus *Polymyxa graminis*. A serological assay with RSNV antiserum, confirmed the emergence of rice stripe necrosis in the Americas.

Activity 6. *Development of disease screening procedures:* The fungus that transmits RSNV, *Polymyxa* spp., has been maintained at CIAT in infested soil obtained in affected rice fields. RSNV transmission experiments have been conducted: 1) planting rice in soils collected from the rhizosphere of RSNV-infected plants grown under field conditions; 2) contaminating soil with dehydrated root extracts obtained from RSNV-infected rice plants, and 3) micro-manipulation of the resting structure (cystosorus) of the fungus, to inoculate disease-free rice seedlings. These preliminary tests have been conducted at CIAT in anticipation of the more in-depth study on the biology of the fungal vector of RSNV in the tropics, to be conducted at Rothamstead under the direction of Dr. Michael Adams. In the mean time, it has been possible to transmit RSNV to healthy rice plants, and to maintain the virus and fungus vector in sterile sand contaminated with spore suspensions and dehydrated root powder obtained from RSNV-infected rice plants. The use of contaminated soil has yielded variable results, as determined by highly variable disease incidence rates in artificially-inoculated rice seedling lots.

Activity 7. *Diagnostic techniques* *:* Leaf extracts and partially purified preparations from infected rice plants were negatively stained in 2% uranyl acetate, pH 3.7, and examined with a transmission electron microscope. The frequency distribution of the lengths recorded for a total of 158 particles observed, were graphed as a histogram. Leaf tissue of symptomatic rice plants was prepared for cytology by preliminary fixation with half-strength Karnovski fixative for 24 hr at 4 C, followed by a secondary fixation in 1% osmium tetroxide for 1 hr at room temperature. After dehydration in a graded series of alcohol and acetone, the fixed tissue was embedded in the low-viscosity epoxy resin medium of Spurr. Thin sections were made with a diamond knife using an ultramicrotome. Scanning electron microscopy of fungal structures was performed according to the method of Wynn . For light microscopy, roots of symptomatic rice plants were washed with sterile water, and then stained 15 min in 0.05% cotton blue prepared in lactophenol.

All symptomatic rice plants were shown by electron microscopy to contain rod-shaped particles ca. 20 nm in diameter (Fig. 1) with a bimodal length distribution of 260 and 360 nm. These particles were not observed in symptomless rice plants. Partially purified preparations (Fig. 2), exhibited a typical nucleo-protein spectrum with a maximum ultraviolet absorbance peak at 260 nm, and an $A_{260/280}$ ratio of 1.5 (uncorrected for light scattering). In transmission electron microscopy examinations, the cytoplasm of infected rice cells contained particle aggregates observed in longitudinal or cross section (Fig. 3A). The disease also induced noticeable cytopathic changes in infected cells, particularly affecting mitochondria (Fig. 3A) and the endoplasmic reticulum (Fig. 3B).

The examination of the root system by light microscopy revealed the presence of abundant cystosori (Fig. 4) containing irregular aggregates of spores (Fig. 4) characteristic of *Polymyxa* spp. vectors of plant viruses .

An antiserum produced against the African isolate of RSNV was used to positively identify the Colombian isolate of RSNV, by serologically specific electron microscopy and Western Blotting. These diagnostic techniques are highly sensitive and can be used to detect the virus in infected plants without any previous virus concentration procedure.

Activity 8. ***Survey of rice producing regions in Colombia and Latin America.*** As shown in Figure 1, RSNV has been detected in the main rice-growing departments of Colombia, namely, Tolima, Huila, Meta, Casanare, Cauca, Cundinamarca, Cordoba and Antioquia. The virus has not yet been detected in two important rice-producing departments in the northern coast of Colombia: Cesar and Guajira.

In 1997, the disease survey was conducted at the epicenter of the first RSNV outbreak, namely, the Departments of Meta and Casanare. A preliminary map is included (Fig.) showing the areas of high disease incidence or "hot spots". This information is being used to create a geographic information system (GIS) for rice stripe necrosis in Colombia, linking the distribution of the disease to potential epidemiological parameters, such as climate, soil characteristics, water sources and cultural practices in affected regions and, particularly, in "hot spots".

The survey of rice production areas in Latin America is being conducted in collaboration with the Latin American Fund for Irrigated Rice (FLAR), which groups the main rice producing countries in Latin America. The members of FLAR are very concerned about the potential threat of rice stripe necrosis to rice production in the region and, consequently, the Fund financed last year the publication of an informative leaflet about the disease , its diagnosis and tentative control methods. The leaflet provides rice growers, technicians and professionals with adequate information to prepare samples for laboratory analyses. So far, we have received reports from Nicaragua and Argentina, regarding the observation of similar symptoms in commercial rice fields in those two countries. Argentine scientists have observed fungal structures in roots, similar to those reported for *Polymyxa,* but the virus has not been detected by electron microscopy in affected rice plants. No samples have been received from Nicaragua for diagnostic purposes. (only a sterile, 1 cm2 piece of dry leaf tissue from a symptomatic plant is necessary for electron microscopy). Should this situation persists, it would facilitate the implementation of phytosanitary methods to prevent the introduction of RSNV and its vector into other rice-growing countries of Latin America.

Activity 9. ***Survey of rice-producing regions in West Africa.*** Despite the fact that rice stripe necrosis was first detected by French scientists in Ivory Coast, the pathologist (Dr. Abdoul Aziz Sy) of the West Africa Rice Development Association (WARDA), located in Bouake, Ivory Coast, was not aware of the presence of this viral disease in this country. Information regarding the original distribution of RSNV in Ivory Coast, was then sought directly from Dr. Claude Fauquet, the French virologist who described this virus from W. Africa (currently at the Scripps Institute, California, USA). Dr. Fauquet commented that the virus was widely distributed in Ivory Coast, and suggested the name of Dr. Placide N'Guessan at the Institut des Savanes (IDESSA), Bouake, Ivory Coast, as a contact person. Dr. Guessan quickly confirmed the presence of RSNV at their main station and in farmers' fields, particularly during 1995. Late last year, Dr. David Johnson, a weed specialist and ODA project scientist stationed at WARDA visited CIAT. Dr. Johnson was informed of the project and earlier negative contacts with WARDA regarding RSNV. He was quite surprised to hear about this disease and, upon being shown pictures of the characteristic symptoms induced by RSNV, he concluded that this disease had been present for several years at WARDA's headquarters, affecting his trials and those of other scientists, but that nobody knew about the nature of this problem. The presence of RSNV at WARDA was confirmed by electron microscopy this year, and WARDA's scientists have taken measures to control the disease on campus.

Dr. David Johnson of WARDA organized a meeting among cropping systems and IPM specialists, and rice breeders working in West Africa, regarding the occurrence of RSN-like symptoms in the region. The following is a summary of the enquiry conducted by Dr. Johnson:

Table 1. Probable Distribution of Rice Stripe Necrosis Virus in West Africa

COUNTRY	RSN-SYMPTOMS	FIRST OBSERVED	ECOLOGY
Benin	Observed	Mid 80's	Low and upland
Burkina Faso	Not observed		
Ghana	Observed	1990	Hydromorphic
Guinea	Observed	1990's	Low and upland
Nigeria	Observed	1993	Upland
Mali	Not observed		
Sierra Leone	Observed	1976	Upland
Chad	Not observed		
Togo	Observed	1994	Low and upland
Senegal	Observed	1994	Lowland

It is worth mentioning that by 1983, the probable distribution of RSNV in West Africa already included Liberia, Nigeria and Sierra Leone, besides Ivory Coast (according to C. Fauquet). WARDA is currently looking for a new pathologist to lead its IPM group, as Dr. Sy left his position this year. As soon as this position is filled, we expect to conduct some collaborative activities in W. Africa, to better document the RSNV situation in that region.

OUTPUT 4. PROJECT PRIORITIES AND RESEARCH CAPACITY ENHANCED

A. TECHNICAL REPORT 1997. FLAR GENETICS

James W. Gibbons

The basic research objectives of FLAR are: continue with access and interchange of elite germplasm and information through the INGER-LAC network; develop and characterize parents for the use of regional temperate and tropical breeding programs; develop new rice cultivars with stable resistance to the principle stresses in the region; investigate post-harvest aspects of grain quality and milling; and promote strategies of crop management for a more sustainable rice culture. This report will focus on progress toward the breeding objectives of FLAR.

Santa Rosa

FLAR continues to utilize the Santa Rosa Experiment Station in Villavicencio, Colombia, as it's primary breeding site. The high natural incidence of rice blast disease insures confident selection and characterization of local and introduced genetic materials. To insure a uniform pressure for blast, "spreader rows" of defeated commercial cultivars are planted in perpendicular rows several weeks prior to the test material. The breeding lines are mixed 50/50 with the highly susceptible cultivar "Fanny" prior to seeding. Evaluations are made for the disease in the leaf and neck stages of the disease. Due to the high and uniform disease levels, a range of reactions results which facilitates targeting of test lines to specific agroecosystems. In 1997, 3328 experimental lines and populations were planted in Santa Rosa (Table 1). This material was composed of lines from the 1997 Acarigua workshop, introductions from IRRI, Thailand, and CIRAD, in addition to populations F2 to F6. Table 2 shows the segregating materials which are in the FLAR germplasm flow. The selections from F4-F6 and anther culture derived R2 populations are included in the new VIOFLAR (International Observational Nursery – FLAR) to be distributes to FLAR member countries in 1997-98. The selected F2 populations for the temperate and tropical regions are given in Tables 3 and 4. These selections will be evaluated for Hoja Blanca and Tagosodes tolerance for the tropical lines and quality for all selections. Those not meeting standards will be discarded with the remaining ones advanced for planting at Santa Rosa in 1998.

Crossing Program:

In 1997 we have processed more than 300 triple crosses and selected more than 4700 F2 populations for planting in Santa Rosa in 1998A. Of 190 crosses destined for the temperate zones, about 126 have been passed through the process of doubled haploid breeding in cooperation with the CIAT biotechnology unit. As of October 1997, we have about 2700 R1 lines originating from 40 triple crosses in the field for selection. We have programmed about 785 triple crosses for the tropical zone of which 135 are specifically requested by FEDEARROZ of Colombia and 130 for FUNDARROZ of Venezuela.

Parental Lines:

During the last two years we have added a total of 147 new parents in the FLAR working germplasm collection (Table 5). These accessions have been characterized for morphological and physiological traits such as height, days to 50% flowering, vegetative vigor, grain quality, blast, brown spot, leaf scald, grain discoloration, Hoja Blanca, and Tagosodes. This information is critical for the successful programming of crosses which result in lines adapted to the different environmental conditions of Latin America.

Grain Quality and Milling:
Considering that the grain quality of rice is of up most importance in the market, FLAR continues providing laboratory analyses to the members of FLAR. In 1997, our laboratory analyzed more than 11,000 samples for grain length, white belly, and gelatinization temperature (Table 6). The chemical analysis of amylose content and the mechanical analysis of milling quality require more time so that only the most advanced lines receive these tests. Even with these limitations, more than 6,600 lines were evaluated for amylose content and 431 lines for milling quality this year. The experimental lines which do not meet quality standards will be discarded in early generations.

Hoja Blanca Virus Disease:
The disease caused by the Hoja Blanca Virus and its insect vector *Tagosodes orizicolus* is endemic to the tropics of Latin America and the Caribbean. In Colombia and other tropical countries the incidence of the disease has been increasing. Integrated crop management and the use of resistant varieties is the best method of control for this disease. In cooperation with CIAT scientists we are selecting breeding lines with tolerance to both the virus and the vector. During the first semester of 1997, we evaluated more than 4200 lines reaction to the virus under field conditions and more than 2600 lines for mechanical damage to the insect in the greenhouse (Table 7). Only those lines which are rated as tolerant both to the virus and insect are advanced and distributed to the countries, or included in the breeding program.

Cold Temperatures:
In collaboration with IRGA and Dr. Zaida Lentini of CIAT, we continue our breeding efforts to combine sources for cold tolerance with sources for tolerance to other stresses such as blast and iron toxicity. We use anther culture to obtain doubled haploid lines which are then screened for diseases in Colombia and cold in the south of Brazil. In collaboration with the University of Rio Grande do Sul, we are developing simplified screening methods using conventional and molecular techniques.

Iron Toxicity:
The method which we developed last year for screening, iron toxicity tolerance in concrete tanks has resulted in some inconsistencies in evaluations. We, therefore, are adjusting this method to result in more reliable results.

Cultivars:
The experimental line FB0007 (FB0007-3-1-6-1-M) was selected from a group of 22 lines from FEDEARROZ (Colombian Rice Federation) which were being multiplied by FLAR (Fund for Latin American Irrigated Rice) for rapid inclusion in Colombian regional trials. It was selected due to it's high level of resistance to the Rice Hoja Blanca Virus (VHB) and the insect vector, *Tagosodes orizicolus.* FB0007 derives from the cross Oryzica Llanos 4/P 1274-6-8M-1-3M-1 made in 1992 at Bosconia in the North Coast of Colombia.

It is a late season variety with moderately erect plant type, large panicles and dark green foliage. It yields well in comparison to Colombian check cultivars, and has acceptable long grain appearance and cooking qualities. In extensive tests in the major rice growing areas of Colombia it has shown milling quality results similar to CICA 8, the cultivar which it should replace. Under high disease pressure at the Santa Rosa plant breeding site it has shown a moderate level of resistance to blast disease, and acceptable levels of tolerance to leaf scald and grain discoloration. Breeder and Foundation seed of this line are being produced at CIAT Headquarters under the supervision of FEDEARROZ breeders and will be available to commercial seed producers at the end of 1997 or early 1998.

Rice Plant Breeders Course:
Following the recommendations of FLAR's technical committee, a course in plant breeding was held in Palmira and Villavicencio, Colombia during July 1997.

Objectives:

A. Become knowledgeable of the different activities and developments in plant breeding and how to use them to solve problems in the different FLAR countries.
B. Promote the interchange of ideas among the participants.
C. Define the flow of germoplasm among the FLAR member countries.
D. Standardize methodologies of evaluation and selection of experimental lines.

Activities:

A.Palmira, 7 to 11 July, 1997

Evaluations
- Hoja Blanca and Tagosodes
- Blast Linages
- Iron Toxicity
- Quality Laboratory
- Milling Lab

Methods
- Germplasm Bank
- Crosses
- Breeders Seed Production
- Anther Culture
- Biotechnology
- Data Base Management

B. Villavicencio, 14 July to 1 August
- Field Design Methods
- Management of Experimental Materials
- Germplasm Evaluation for Agronomic and Biological Traits
- Selection of Germplasm
- Presentations of Participants
- Germplasm Flow Definition
- Visits to Seed Companies, Rice Mills, and Commercial Rice Fields

Participants:

- Ing. Sergio Gindri Lopez - IRGA, Brazil
- Ing. Juan Sierra - FEDEARROZ, Colombia
- Ing. Alejandro Vargas - FEDEARROZ, Colombia
- Ing. Evelyn Quiroz - FEDAGPA, Panamá
- Ing. Marco Acevedo - FUNDARROZ, Venezuela
- Ing. Jaime Florez - Colciencias, Colombia
- Ing. Rosa Alvarez - FUNDARROZ, Venezuela
- Ing. Daniel Parzajuk - APAI, Paraguay
- Ing. Osvaldo García - FINSA, Guatemala

Results:

The participants were exposed to a wide range of breeding tools and experiences thanks to the cooperation of all the scientists of CIAT and FLAR. They were exposed not only to the Colombian rice situation but also to the experiences of each country represented. There was sufficient time for the gaining of much knowledge and experience of field evaluation methodologies. With this experience the participants selected a wide range of rice experimental lines, which was dispatched to them in their respective countries for further testing (Table 8).

TABLE 1. Material Planted at Santa Rosa, Colombia, 1997A.

Class of Material	Total Lines Evaluated
1 COMMERCIAL CULTIVARS	237
2 VIOAL 1997 (Preliminary)	126
3 F4FLAR	187
4 F5F6-INGER	689
5 F5F6-FLAR	36
6 CUBA	324
7 VENEZUELA	260
8 FEDEARROZ	66
9 USA	52
10 GUAYANA	2
11 SURINAM	50
12 VIARC-96	20
13 NTP	446
14 F6F3TAIL	104
15 TOL-FRIO	97
16 R2-FLAR	463
17 AROMATICOS	30
18 BRASIL-IRGA	60
19 COSTA RICA	99
20 CORPOICA	45
21 FEDE-MONTERIA	351
22 PROGENITORES	136
23 LIB-OBSERV.-ICA	356
24 F4-CORPOICA	266
25 LINEAS F3-FEDEARROZ	348
26 PROMISORIAS	60
27 F2-FLAR	546
28 F2-FEDEARROZ	163
TOTAL	3348

TABLE 2. FLAR Germplasm Planted and Selected at Santa Rosa Experimental Station, Colombia, 1997 A.

Material	Number of Lines			New Families
	Evaluated	*Selected*	*%*	
F2	545	46	8.4	634
F4 - F6	912	220	24.1	
Anther Culture	463	81	17.5	
TOTAL	1920	347	18.1	634

TABLE 3. Temperate F2 Populations Selected in Santa Rosa, Colombia, 1997A.

reg	ncampo97a	pedigree	vg	flor	bl1	bl2	bl3	nbl	bs	lsc	gd	seleciones
1	974837	FL00001-1	5	76	1/3	1/3	1/3	3	1	1	1	13
2	974844	FL00001-8	5	79	1/2	1/3	1/3	1	1	1	1	10
3	974847	FL00001-11	5	80	1/2	1/4	1/4	1	3	1	1	4
4	974848	FL00001-12	5	84	1/3	1/4	1/4	1	1	1	1	15
5	974851	FL00001-15	5	86	1/3	1/4	1/4	1	1	3	1	18
6	974858	FL00001-22	5	82	1/3	1/3	1/3	1	1	1	1	14
7	974860	FL00001-24	3	78	1/3	1/3	1/3	1	1	3	1	20
8	974866	FL00001-30	5	78	1/2	1/4	1/4	1	1	1	1	37
9	974930	FL00003-13	3	76	1/3	1/3	1/3	1	1	1	1	8
10	974933	FL00003-16	3	74	1/3	1/3	1/3	3	1	1	1	12
11	974934	FL00003-17	1	74	1/3	2/3	2/3	3	1	1	1	9
12	974941	FL00004-1	3	79	1/3	1/3	1/3	1	1	1	1	10
13	975003	FL00005-17	3	85	1/4	1/4	1/4	1	3	3	1	8
14	975010	FL00005-24	3	90	½	1/4	1/4	3	1	3	1	8
15	975014	FL00005-28	3	80	1/3	1/4	1/4	1	1	1	1	4
16	975044	FL00006-9	1	70	1/1	1/2	1/2	3	3	1	1	21
17	975068	FL00007-17	1	84	1/3	2/3	2/3	3	1	1	1	17
18	975070	FL00007-19	1	76	½	1/4	1/4	1	1	1	1	18
19	975072	FL00007-21	3	79	2/3	1/4	1/4	3	1	1	1	15
20	975083	FL00007-32	3	86	1/3	2/3	2/3	3	1	3	1	14
21	975097	FL00007-46	5	82	½	1/4	1/4	3	1	3	1	7
22	975165	FL00106-2P	3	83	½	1/3	1/3	1	1	1	1	12

TABLE 4. Tropical F2 Populations Selected in Santa Rosa, Colombia, 1997A.

Reg.	NCampo97A	Pedigree	VG	Flor	BL1	BL2	BL3	NBL	BS	LSC	GD	Selecciones
1	975221	FL00135-1P	3	92	1/4	1/4	1/4	1	1	1	1	20
2	975232	FL00138-3P	3	86	1/3	1/4	1/4	1	1	1	1	16
3	975240	FL00140-1P	5	74	1/2	1/3	1/3	3	1	1	1	3
4	975241	FL00140-2P	5	74	1/2	1/3	1/3	3	1	1	1	7
5	975261	FL00141-20P	1	90	1/4	1/4	1/4	1	1	1	1	21
6	975271	FL00144-1P	1	84	2/3	2/3	2/3	1	1	1	1	32
7	975272	FL00144-2P	1	90	1/3	1/4	1/4	3	1	1	1	6
8	975273	FL00144-3P	3	86	1/4	1/4	1/4	1	1	1	1	14
9	975276	FL00144-6P	3	82	1/2	1/4	1/4	1	1	1	1	11
10	975278	FL00144-8P	3	90	2/2	1/4	1/4	1	1	1	1	22
11	975281	FL00147-1P	3	92	1/3	1/4	1/4	1	1	1	1	11
12	975282	FL00147-2P	3	86	2/3	1/4	1/4	1	1	1	1	11
13	975284	FL00147-4P	1	90	1/3	1/4	1/4	1	1	1	1	10
14	975288	FL00147-8P	3	86	2/3	1/4	1/4	3	1	1	1	15
15	975290	FL00147-10P	3	89	1/3	1/4	1/4	1	1	1	1	14
16	975291	FL00147-11P	3	91	1/3	1/4	1/4	1	1	1	1	15
17	975293	FL00147-13P	3	102	1/4	1/4	1/4	1	1	1	1	16
18	975333	FL00159-6P	3	90	1/2	1/4	1/4	3	1	1	1	15
19	975338	FL00162-1P	1	86	1/3	1/4	1/4	3	1	3	1	6
20	975359	CT9736-1P	1	86	1/1	1/3	1/3	1	1	1	1	11
21	975360	CT9736-2P	1	78	1/1	1/4	1/4	1	1	1	1	11
22	975367	CT9736-9P	1	76	1/3	1/3	1/3	1	1	1	1	27
23	975370	FL00364-1P	1	86	1/2	1/3	1/3	1	3	1	1	5
24	975381	FL00365-2P	3	84	2/3	1/3	1/3	1	1	1	1	11

TABLE 5. New Accessions in the "Working Collection", 1997.

BANCOWC	PEDIGREE	HT	FL	VG	BL	NBL	BS	LSC	GD	HB	SOG	TG	CB	LG
WC/324	IA CUBA 19		110	3	4			1		7		AI	3.0	1
WC/325	IA CUBA 22		104	5	7	7	1	1	3	9		B	1.0	1
WC/326	IA CUBA 17		112	3	3			1		9		IB	2.0	3
WC/327	IA CUBA 23		106	3	5	5	1	1	3	9		I	2.0	3
WC/328	CT10004-4-3-1P-1-2		104	5	4	3	1	1	3	9		I	1.0	3
WC/329	CT10344-7-8-2P-2-2		90	3	5	3	1	1	1	7		BI	0.4	3
WC/330	CT11014-10-1-2		104	3	4	1	1	3	3	7		A	0.6	3
WC/331	CT9497-4-3-1-1-M-4-3P-M		104	5	7	5	3	3	3	7		B	1.6	3
WC/332	CT10175-5-1-3P-1-3-2P		104	3	5	3	1	3	3	3		BIA	1.0	3
WC/333	CT10323-29-4-1-1-1T-2P		102	3	3	1	1	3	1	1		B	0.8	3
WC/334	FB0007-3-1-6-1-M		104	1	4	1	1	1	1	*		BI	1.0	3
WC/335	CT9682-2-5-1-2-1	104	96	3	4	3	1	1	1	5		B	2.0	3
WC/336	CT9809-7-1-M-1-1	101	96	3	6	5	1	1	3	*		B	0.8	3
WC/337	CT9506-38-5-1P-4PT	98	88	3	5	7	1	1	1	3		I	1.0	3
WC/338	CT9737-1-1P-2-1	108	93	3	4	5	1	3	1	9		I	0.4	3
WC/339	CT9159-18-2-3-1	110	90	1	5	5	1	1	3	3		B	0.6	3
WC/340	CT10335-11-8-1P-4-3P		104	3	5	3	1	3	3	*		I	1.0	3
WC/341	CT9162-12-6-2-2-1	87	96	1	4	1	1	1	3	*		BI	0.4	3
WC/342	CT9509-28-3-3P-M-1	95	94	3	5	3	1	1	1	3		BI	0.6	3
WC/343	CT9506-28-3-3P-M-1-M	96	100	3	5	3	1	3	1	3		I	1.4	3
WC/344	CT9824-9-3-1P-2-M		118	3	5	1	3	1	5	3		BI	0.6	1
WC/345	CT11891-2-2-7-M	105	66	3	2	3	1	3	1	1		I	1.0	3
WC/346	CT9155-2-3-1-2M-4-1P	110	83	1	5	5	1	1	1	9		B	1.4	3
WC/347	CT9737-8-9-1-1-1P	97	83	1	5	9	1	1	3	9		B	0.6	1

TABLE 5. New Accessions in the "Working Collection", 1997.

BANCOWC	PEDIGREE	HT	FL	VG	BL	NBL	BS	LSC	GD	HB	SOG	TG	CB	LG
WC/348	CT8008-3-5-1P-M	99	76	1	3	3	1	1	1	9		I	0.4	3
WC/349	CT9980-25-3-6-CA-1M	106	98	3	3	3	1	1	3	9		I	2.0	3
WC/350	CT10588-CA-1M	112	83	3	4	3	1	3	1	9		A	1.0	3
WC/351	CT10865-CA-12M	93	86	3	4	1	1	3	1	9		B	1.2	3
WC/352	CT10871-1-CA-1M	100	86	1	4	3	1	3	1	9		B	1.6	3
WC/353	CT11685-7-F4-6-2P-1	118	84	3	4	3	3	3	3	1		B	1.6	5
WC/354	CT11691-17-F4-1-1P-2	112	84	3	5	3	1	3	3	9		I	1.8	3
WC/355	CT11696-9-F4-10-2P-3	114	86	3	4	1	1	3	1	7		I	0.6	3
WC/356	CT11782-2-F4-3-1P-3	121	73	5	5	1	3	1	1	1		I	0.6	3
WC/357	CT11783-14-F4-1-1P-1	125	88	3	4	5	1	1	3	1		B	1.2	3
WC/358	CT11800-22-F4-1-1P-1	121	83	3	4	5	1	1	1	5		BI/A	1.0	1
WC/359	VSTA/LBNT//RSMT	81	81	3	3	5	3	1	3	9		I	1.0	3
WC/360	VSTA/LBNT//L 201/3/SKBT	92	72	5	4	9	3	1	3	9		I	1.0	1
WC/361	82CAY21/LMNT//L 202	68	74	3	5	9	3	1	3	9		I	1.0	3
WC/362	NWBNT/KATY/RA73/LMNT	96	74	5	5	9	5	5	3	9		A	0.8	5
WC/363	GFMT*2/TQNG	88	80	3	4	5	3	3	3	9		I	2.0	3
WC/364	LBNT/STBN//NWBT/3/MILL	92	74	5	3	7	3	1	3	9		I	1.6	3
WC/365	CT8008-16-31-3P-M		104	3	6	3	7	1	3	9		I	0.6	1
WC/366	IR65598-27-3-1	89	98	5	5	1	5	5	5	9		I	3.6	7
WC/367	IR65600-96-1-2-2	---	---	0	0	---	---	---	---	9				
WC/368	IR66155-2-1-1-2	93	90	5	4	5	1	5	3	7		A	3.8	7
WC/369	CT9682-2-M-14-1-M-1-3P-M-1	98	98	1	4	1	1	1	3	*		I	0.4	3
WC/370	CT10554-4-4-2-2-M	96	83	3	4	3	1	1	5	9		B	0.8	3
WC/371	CT10166-16-1-2P-1-3	92	88	1	4	5	1	1	5	*		B	1.0	3

TABLE 5. New Accessions in the "Working Collection", 1997.

BANCOWC	PEDIGREE	HT	FL	VG	BL	NBL	BS	LSC	GD	HB	SOG	TG	CB	LG
WC/372	CT9509-17-3-1-1-M-1-3P-M-1	90	85	1	3	3	1	3	1	3		B	1.4	3
WC/373	CT10825-1-2-1-3-M	99	90	5	7	7	1	1	1	3		B	0.4	3
WC/374	CT11008-12-3-1M-4P-4P	101	87	1	3	3	1	3	5	*		I	1.0	3
WC/375	CT10310-15-3-2P-4-3	91	90	1	2	3	1	3	3	3		I	0.6	3
WC/376	IRGA 234-21-5-6-1	86	90	1	3	3	1	3	3	9		B	0.6	3
WC/377	IRGA 660-3-13-5-3	80	77	1	4	5	1	1	3	9	3	B	0.6	3
WC/378	IRGA 659-1-2-2-2	95	82	3	2	1	3	3	3	7		BI	0.6	3
WC/379	IRGA 411-1-6-1F-A	100	84	3	4	5	3	1	3	9		B	1.0	3
WC/380	IRGA 284-18-2-2-2	92	76	1	5	9	1	1	3	9		B	0.6	3
WC/381	IRGA 370 -42-1-1F-C-1	88	77	3	5	7	1	1	3	7		B	0.6	3
WC/382	IRGA 369-31-2-3F-A1-1	89	78	3	6	9	3	1	5	7		B	0.4	3
WC/383	IRGA 440-22-1-3-2	98	84	1	4	1	1	1	3	9		B	0.4	3
WC/384	CNAx 5011-9-1-6-4-B	102	80	1	5	5	1	1	3	9		BI	0.4	1
WC/385	CT9882-16-4-2-3-4P-M	90	90	3	6	1	1	1	1	9		B	0.8	3
WC/386	CT9868-16-3-1-2-3P-M	104	94	1	4	1	1	1	1	7		I	0.4	3
WC/387	CT8455-1-13-1-M-2P	104	83	3	2	1	1	1	1	3		B	1.0	3
WC/388	CT8665-1-1-1P-4	98	90	3	2	3	1	1	3	9		IB	0.2	3
WC/389	CT8248-1-12-1P-M-P	93	85	1	4	1	1	1	1	3		B	0.6	3
WC/390	CT8452-2-16-3P-M	93	87	1	4	3	1	1	1	9		B	0.2	3
WC/391	CT8285-13-4-1P-M	91	98	3	5	5	1	1	1	9		B	0.2	3
WC/392	PNA 1005-F4-171-3-1	103	96	1	5	5	1	1	1	9		B	0.6	3
WC/393	ECIA 38-2-4-2-5-6	94	98	1	4	3	1	1	1	9		I	0.2	1
WC/394	CT10184-2-1-M-1-MI	100	100	3	5	1	1	1	1	3		I	0.4	3
WC/395	CT6163-8-9-5-2-M-85-M		---	0	0	---	---	---	---	3		B	0.4	3

TABLE 5. New Accessions in the "Working Collection", 1997.

BANCOWC	PEDIGREE	HT	FL	VG	BL	NBL	BS	LSC	GD	HB	SOG	TG	CB	LG
WC/396	CT8240-1-5-2P-M-1P	86	86	3	5	7	1	1	1	9		I	0.6	3
WC/397	P 5746-18-11-1-2-2A-1BRH	90	96	1	5	5	1	1	1	3		B	0.8	3
WC/398	CT11275-3-F4-8P-2	106	94	1	3	3	1	3	3	3	1	B	0.6	1
WC/399	CT11299-4-F4-18P-4	97	92	1	2	1	1	3	1	*	5	I	0.2	3
WC/400	CT11280-2-F4-12P-5	103	88	1	2	1	1	1	1	3	9	IA	0.4	1
WC/401	IR61987-51-3-3	94	80	3	4	3	1	1	3	3		A	1.0	3
WC/402	IR62061-89-1-3-2	88	88	1	4	5	1	1	3	*		A	2.6	3
WC/403	CT8709-4-4-5P-2-M	98	96	1	4	5	1	3	1	7		B	0.8	3
WC/404	CT8753-6-8-7P-2-M	110	98	3	3	1	1	3	1	9		I	2.6	3
WC/405	CT9748-3-1-1P-2-M		110	3	4	1	1	5	3	*		A	0.6	3
WC/406	CT11008-12-3-1M-1P-4P	98	91	1	3	3	1	3	3	*		I	1.2	3
WC/407	CT9682-M-10--2-M-1-1P	---	---	0	0	---	---	---	---	-				
WC/408	CT8008-16-10-10P-M	92	100	1	6	5	1	3	1	9		I	0.4	1
WC/409	CT8008-3-5-8P-M-2P	95	83	1	4	5	1	3	3	9		I	0.6	1
WC/410	CT8945-8-17-2T-M	92	98	1	2	3	1	3	3	3		B	1.8	3
WC/411	PR23613-1-4	91	82	1	2	1	1	1	1	1		B	2.4	3
WC/412	CT9841-5-2-1P-2I-2I-M	90	85	3	3	7	1	3	3	3		B	0.6	3
WC/413	CT9748-13-2-1-M-M-1-1	102	93	3	3	5	1	1	1	3		BI	0.4	3
WC/414	CT10491-12-4-2T-3P-1P-3-1X	102	87	3	5	9	1	1	1	3		IB	0.6	3
WC/415	CT11030-1-2-2T-1P-1P-3-1X	97	91	3	5	7	1	1	1	3		IB	0.4	3
WC/416	CT11032-2-4-3T-3P-3P-1-1X	104	90	1	5	7	1	1	1	1		I	0.2	3
WC/417	CT11260-7-F4-13-4-1X		104	1	4	3	1	5	1	3		I	0.8	3
WC/418	CT10310-15-9-2P-3-1T-2P-1X	98	87	1	4	9	1	1	3	3		B	0.4	3
WC/419	CT10323-8-2-2P-1-1T-4P-4P-1X	100	93	1	2	1	1	1	3	5		B	2.2	3

TABLE 5. New Accessions in the "Working Collection", 1997.

BANCOWC	PEDIGREE	HT	FL	VG	BL	NBL	BS	LSC	GD	HB	SOG	TG	CB	LG
WC/420	CT11026-3-9-1T-2P-3P-1-1X	102	92	1	4	7	1	1	1	3		BI	0.2	3
WC/421	FB0100-10-1-M	103	98	3	5	5	1	1	1	7		B	0.8	3
WC/422	CT10175-4-6-2P-2-2	104	100	3	5	5	1	1	1	*		I	0.4	3
WC/423	CT10194-5-1-3-2T-1-1	106	104	3	5	1	1	3	3	*		B	0.2	3
WC/424	CT10240-10-1-2-1T-2-1	106	104	3	5	1	1	1	1	3		B	0.2	3
WC/425	CT10166-2-1-1T-1C	92	87	1	5	1	1	1	1	9		B		
WC/426	CT10175-5-10-3P-5-3	104	93	1	4	7	1	1	3	3		B	0.6	3
WC/427	CT10244-1-1-1-1T-2-1	104	98	3	5	7	1	1	1	7		B	0.6	3
WC/428	CT10325-29-4-1-1T	102	93	3	5	5	1	1	1	7		B	0.2	3
WC/429	CT10175-6-6-2P-3-M	106	93	3	6	3	1	3	1	3		B	0.6	3
WC/430	CT9506-18-7-1T-2	93	100	3	6	3	1	1	1	3		B	0.2	3
WC/431	CT9886-3-1E-1-5-4P	97	98	3	5	3	1	1	1	7		B	0.6	3
WC/432	IR62140-91-2-2-2-2-3	100	94	3	4	5	1	3	1	7		I	1.8	3
WC/433	PSB RC-2	90	104	3	2	3	1	3	3	5		IA	0.4	3
WC/434	CT8285-8-8-2P-M-1P-12		84	3	4	1	1	1	1	*				
WC/435	RCN-B-93-083	92	84	5	5	7	1	1	1	9	3	I	2.2	1
WC/436	CT10323-16-2-1-1-1	103	96	1	5	3	1	3	1	*		B	0.4	3
WC/437	CR 1515													
WC/438	CT9157-3-2-6-2		96	5	1	1	1	1	1	9				
WC/439	CNARR4949-8B-BM85-15-2P	82			1	1	1	3	1					
WC/440	CT11256-5-F4-28P-5P	89			3	1	1	3	3					
WC/441	CT11424-14-F4-12P-1P	97			2	1	1	5	5					
WC/442	CT12908-1-4-9-2-M	96			4	1	1	3	1					
WC/443	CT10355-10-4-1T-2P-2P-2	105			4	1	1	1	1					

TABLE 5. New Accessions in the "Working Collection", 1997.

BANCOWC	PEDIGREE	HT	FL	VG	BL	NBL	BS	LSC	GD	HB	SOG	TG	CB	LG
WC/444	CT11260-11-F4-5P-3	94			1	1	1	1	1					
WC/445	CT10166-1-1E-3P-6-2-2P-5P	100			1	1	1	1	1					
WC/446	CNAx 5013-13-2-2-4-B	94	94	3	1	1	5	1	3	1		IB	0.6	1
WC/447	CNARR4949-8B-BM85-15-1P	90	89	3	1	1	1	1	1	1		B	1.6	1
WC/448	CT9145-4-21-5P-1-MI-F8-3P	90	94	3	1	1	1	1	1	1		B	1.6	3
WC/449	CT11361-17-F4-11P-1P	100	96	3	1	1	1	1	3	1	7	B	0.4	3
WC/450	CT11369-1-F4-17P-4P	88	94	3	1	1	1	1	3	1		AI	1.2	3
WC/451	CT11626-14-4-2-1-M	94	83	5	3	3	1	1	1	1	9	A	2.0	3
WC/452	CT10491-12-4-2T-3P-2P-1	102	94	5	3	5	1	1	3	1		I	1.0	3
WC/453	CT10992-3-4-1T-3P-2P-3	87	85	3	1	1	1	1	3	1		A	1.2	3
WC/454	CT11072-2-4-1T-1P-2P-2	91	110	3	3	3	1	5	5	1		I	1.0	3
WC/455	CT9992-2-7-2T-2P-3P-3	108	90	1	4	7	1	1	1	1	7	B	0.4	3
WC/456	CT8837-1-17-1P-4-M		96	1	3	3	1	1	1	9	3	B	1.0	3
WC/457	CT9868-3-2-3-1-4P-M-1-1P		88	1	1	1	1	3	1	1	7	IA	0.8	3
WC/458	CT9852-3-2-1-2-F7		90	3	2	1	1	1	1	7	3	I	0.2	3
WC/459	CT9509-17-3-1-1-M-1-3P-M-3-3P		87	1	1	1	1	1	1	1	5			
WC/460	CT9868-3-2-3-1-4P-M-1-3P		98	3	2	1	1	3	1	3	3			
WC/461	CT9892-6-2-1E-2-M-2-MI		80	1	1	1	1	1	3	9	1			
WC/462	CT10308-27-3-1P-4-3-2P		94	1	3	3	1	1	1	3	3	B	2.0	3
WC/463	CT10532-1-1-2-1-1T-3P		98	3	2	1	1	1	1	1	3	B	1.2	3
WC/464	CT11256-5-F4-28P-1P	101	83	1	1	1	1	3	3	1		B	0.6	3
WC/465	IR63872-8-3-1-2-1		88		5	7	1	5	5					
WC/466	IR61009-72-2-3-2	88	88	3	1	3	1	1	3	1		AI	1.6	3
WC/467	CT13737-5-5-3P-M	97	80	1	1	1	1	1	3	1				
WC/468	CT12494-14-M-2-2-M	89	83	1	1	1	1	1	3	1		I	2.0	3
WC/469	PANAMA 31-89 (VIOAL 89)		92	1	3	3	1	1	3	5				
WC/470	CT9506-38-M-6-1-M-2-1P(IG 240)		96	1	4	3	1	1	1	9		I	0.2	1

TABLE 6. Analysis of Grain Quality, 1997`- Number of Samples

Origin	L, CB, TG *	Amylose	Milling
FLAR			
Palmira/Sta. Rosa	3.510	2.892	271
Colombia	7.242	3.382	160
Brazil	331	334	
Others	50	50	
Total	**11.326**	**6.658**	**431**

* Grain length White Belly, and Gelatinization Temperature

TABLE 7. Materials Evaluated for Hoja Blanca Virus (VHB) in the field and mechanical damage to *T. orizicolus* in the greenhouse 1997. (First Semester, 1997).

	No. Material Evaluated	*Tolerant (0-1-3)**	*Intermediate 5**	*Susceptible (7-9)**	*Material to Reevaluate*
VHB	4245	2007 (47.3%)	555 (13.1%)	1613 (38.0%)	70 (1.6%)
Tagosodes	2603	1448 (55.6%)	104 (4.0%)	733 (28.2%)	318 (12.2%)

OUTPUT 4. PROJECT PRIORITIES AND RESEARCH CAPACITY ENHANCED

B. FARMERS' QUESTIONNAIRES, RICE TECHNOLOGIES AND COSTS

Monitoring farmers' rice management practices is an essential component in the process of improving technologies. The ultimate client of our research is the farmer who adopts the components that are relevant to his crop management plans. The rice project maintains close collaboration with three countries where monitoting of crop management practices is a routine activity in the research program: Colombia, Venezuela and Rio Grande do Sul (Brazil).

In 1997, an effort was made to improve the database of Portuguesa (Venezuela). We gathered data from 1989 and analyzed possible inconsistencies. The process led to the identification of errors that were corrected. In 1998, we will analyze this set and incorporate a new data set from Guárico, that keeps records since 1990.

Collaboration with FEDEARROZ continues since 1987, when the First Rice Census was planned and executed a year later (1988). CIAT participates directly in the application of questionnaires to gather data for the Central zone of the Cauca Valley. A methodology developed in 1991 to estimate economic efficiency is then used to analyze data (for details, see the 1991 Rice Program Annual Report). During 1997, data collected from 45 farmers in a 1995 survey in the Tolima (Colombia) region were analyzed. The results show a wide spread in practices and in gross margins around the mean values (Table 1). The sample was broken down into deciles, using as ranking variable the gross margins of the operation (gross income minus direct costs). The top decile (four farmers) exhibited gross margins of 70% over the direct costs, while at the other end, the worst four performers showed an average gross margin of 9.5% (Table 2). As a general rule, the most efficient farmers are more intensive in the use of machinery and rely less on labor and on pesticides. It is difficult to generalize much more than that. The average farm size for the top four producers is 8.5 has. while the bottom four producers have 20.5 has on average. Seed density is rather high in all cases (over 200 kg/ha) as this is a common strategy against weeds.
The main constraints in terms of inefficiencies are found in machinery use (old equipment, frequently rented) and in herbicide use. This points at the high priority that the weed management aspect has for these farmers, as weeds are the result of poor preparation, seed densities, herbicide use and many other crop management aspects. For CIAT, this points out at the need to collaborate in identifying germplasm that can be more competitive with weeds. For 1998, we will analyze sets of data from Venezuela (from the two major growing areas in Guárico and in Portuguesa) as well as new data from Colombia.

Brazil is preparing a rice census for March, 1998. We collaborated, during 1997, in the design of the census questionnaire, based on our past experience. The main criterion for the questionnaire is to keep it very simple, addressing a few questions for broad characterization of the sample (Table 3). With the data collected, stratified random sampling will allow to dig deeply into the chracteristics of sampled farmers and its rice growing practices as well as the constraints and inefficiencies.

Collaborators: Myriam C. Duque (IP-4); James Silva (IP-4); Luis R. Sanint (IP-4, FLAR)

Table 1. Preliminary results from the study on economic efficiency by rice farmers in Tolima, Colombia. First Semester 1995

	Average	Minimum Value	Maximum Value
Number of Producers	45		
Seed quantity, kg/ha	264	118	329
Labor, hours/ha	159	74	503
Nitrogen Kgs/ha	180	79	294
Phosphorus - P2O5, Kgs/ha	46	0	107
Potasium – K2O, Kgs/ha	62	0	195
Machinery Hours/ha	13	7	20
Herbicides, lts/ha	4.7	1	10
Insecticides, lts/ha	0.6	0	3
Fungicides, lts/ha	1.3	0	8
Farm size, has.	26.7	0.2	395.0
Yield, kgs/ha, field cond.	6,241	4,424	8,550
Unit cost, Col$/ha	162.30	109.90	227.80
Price, Col$/ha	220.30	203.80	235.19
Gross Margin	58.00	-8.30	109.59

Table 2. Results for the best and worst four farmers in the sample, Tolima, 1995

Top four Farmers	**Best**	**Second**	**Third**	**Fourth**
Seed quantity, kg/ha	157	206	199	250
Labor, hours/ha	190	96	79	148
Nitrogen Kgs/ha	166	168	159	186
Phosp- P2O5, Kgs/ha	0	53	0	0
Potas.– K2O, Kgs/ha	0	69	39	0
Machinery Hours/ha	17	12	10	19
Herbicides, lts/ha	4	2	3	7
Insecticides, lts/ha	1	1	0	1
Fungicides, lts/ha	0	2	1	0
Farm size, has.	0.4	10.9	28	0.5
Yield, kgs/ha, field	8,550	6,996	7,002	7,192
Unit cost, Col$/ha	109.90	122.20	127.80	135.50
Price, Col$/ha	219.50	215.60	215.60	219.50
Gross Margin $/ha	109.60	93.40	87.80	84.00
Bottom four farmers	**Last**	**2nd to last**	**Third**	**Fourth**
Seed quantity, kg/ha	265	234	329	250
Labor, hours/ha	134	98	235	215
Nitrogen Kgs/ha	263	140	147	124
Phosp - P2O5, Kgs/ha	68	30	80	50
Potas.– K2O, Kgs/ha	68	31	63	87
Machinery Hours/ha	7	9	14	12
Herbicides, lts/ha	4	5	10	6
Insecticides, lts/ha	0	3	1	1
Fungicides, lts/ha	4	3	3	4
Farm size, has.	1.1	70	1	10
Yield, kgs/ha, field	4,424	4,599	6,316	6,750
Unit cost, Col$/ha	227.80	219.10	191.10	178.90
Price, Col$/ha	219.50	235.20	219.50	221.10
Gross Margin $/ha	-8.30	16.10	28.40	42.20

Table 3. Suggested format for the 1998, Rice Census, Rio Grande do Sul, Brazil

LEVANTAMENTO ESTATISTICO DA LAVOURA ORIZICOLA E RECADASTRAMENTO DOS PRODUTORES

SAFRA 96 / 97

I. IDENTIFICACAO

1. IDENTIFICACAO DEL PREDIO		
LOCALIZACAO	Municipio	
	Distrito	
	Local	
	Pessoa contacto	
2. IDENTIFICACAO DE PROPRIETARIOS (seleccionar la opción que corresponda)		
2.1 EMPRESA	Nombre de la empresa :	
	Proprietarios :	
	Endereco	
	Municipio	
	CEP	
	Fone	
2.2 PRODUTOR	Nombre	
	Endereco	
	Municipio	
	CEP	
	Fone	

II. CARACTERISTICAS DEL PREDIO

1. Area (Hectáreas)		
Totales del predio	En actividad diferente a Arroz	En Arroz(*) **(Ultima safra)**

* Verificar que la suma de las áreas sobre todas las modalidades de un mismo tema sea igual al valor de la columna Arroz (Ultima Safra)

Hectáreas en ARROZ (*)	**Modalidad**	**TEMA**
	Cultivada por propietarios	**2.TENENCIA**
	Cultivada por arrendatarios	
	Cultivada por aparceros	
	Convencional em línea	**3.SISTEMAS DE CULTIVO**
	Convencional a lanco	
	Cultivo mínimo	
	Cultivo pre-germinado	
	Outros	
	Natural	**4.IRRIGACAO**
	Mecánica eléctrica	
	Mecánica diesel	
	Rio	**5. FONTES DE AGUA**
	Arroio	
	Lagoa	
	Acude	
	Outras	
	Básica- Certificada ou Fiscalizada	**6. SEMENTE**
	Comum Propria	
	Comum Comprada	
	Financiada	**7. FINANCIAMIENTO**
	No financiada	
	Isenta	**8. INCIDENCIA DE ARROZ VERMELHO E PRETO**
	Baixa (1 a 5 plantas /m2)	
	Media (6 a 29 plantas /m2)	
	Alta (30 ou mais plantas /m2)	

II. CARACTERISTICAS DEL PREDIO- Continuación

9. Número de cortes					
10. Producao total (Sc seco e limpo)					
11. Area Sistematizada (Ha)					
12. Armazenagem		Convencional	Capac. estatica (Sc)		
		Silos	Capac. Estatica (Sc)		
13. Mano de Obra	Fixa	Num. de pessoas		meses trabalhados	
	Eventual	Núm. de pessoas		meses trabalhados	

COMENTARIOS :

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Entrevistador : ______________________________

Fecha de la entrevista ______________________________

ANNEX 1

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ANNEX 2

PRINCIPAL AND SUPPORT STAFF

PRINCIPAL STAFF

Luis R. Sanint, *Agricultural Economist, Leader and FLAR Executive Director*
Lee Calvert, *Virologist*
Marc Chatel, *Plant Breeder (CIRAD-CA)*
Fernando Correa, *Plant Pathologist*
James Gibbons, *Plant Breeder, FLAR*
Zaida Lentini, *Plant Breeder, BRU*
César P. Martínez, *Plant Breeder*
Francisco Morales, *Virologist*
Michel Valés, *Plant Pathology (CIRAD-CA)*

SUPPORT STAFF

Associates and Assistants

Luis E. Berrío, *FLAR*
Jaime Borrero, *Genetics*
Maribel Cruz, *Virology*
Myriam Cristina Duque, *Biometry*
Fabio Escobar, *Biotechnology*
Daniel I. González, *FLAR*
María del Pilar Hernández, *Entomology*
Nelly Manosalva de Nivia, *FLAR* *
María Nelly Medina, *Leader*
Adriana Mora, *Genetics*
Yolima Ospina, *Genetics (Villavicencio)*
Gustavo Prado, *Pathology*
James Silva, *Biometry*
Mónica Triana, *Entomology*
Edgar Tulande, *Pathology (Villavicencio)*
Ana Cecilia Velasco, *Virology*

Visiting Scientist

Rosa María Alvarez, *Fundarroz, Venezuela*
Dumar Carrillo, *Colciencias**
Diana Delgado, *Colciencias*
Jorge Luis Fuentes, *CEADEN, IIA*
Julio Eduardo Holguín, *Fedearroz, Colombia*
Catherine Pardey, *Colciencias*
Edwin Restrepo, *Colciencias*
Luis Antonio Reyes, *Fedearroz, Colombia*

Thesis, Ph.D.

Ruben Darío Zárate, *Universidad Nacional, Colombia*
Kennet Seebold, *University of Florida, U.S.A.*
Renata Cruz Pereira, *Univ. Federal Rio Grande do Sul, Brazil*

Thesis, Ms.C.

Luis Eduardo Berrío, FLAR/Universidad Nacional, Colombia
Edgar Corredor, FEDEARROZ/Universidad Nacional, Colombia
Carlos Gamboa, DANAC/UCV-Venezuela
Iván Lozano, CIAT/Universidad del Valle, Colombia

Secretaries

Carmenza Llano
Liz Deira Arango
Clara Inés Sánchez

Technicians and Field Staff

Felix Acosta, *Genetics*
Miguel Acosta, *FLAR*
José Demetrio Arboleda, *FLAR*
María Girlena Aricapa, *Pathology*
Jesús E. Avila, *Physiology*
Jairo Barona, *Leader*
Marco Tulio Castillo, *FLAR*
Efrén A. Córdoba, *Entomology*
Gerardo A. Delgado, *Genetics*
Jaime Gallego, *Pathology*
Jairo García, *Genetics*
Aldemar Gutiérrez, *FLAR*
Jorge Ignacio Hernández, *FLAR*
Victoria Eugenia Kury, *FLAR*
Elsy Lasprilla, *Genetics*
Luis Armando Loaiza, *FLAR*
Víctor Hugo Lozano, *ODA*
María C. Martínez, *Virology*
Fabián Mina, *FLAR*
Jaime Morales, *FLAR*
Mauricio Morales, *Entomology*
Rodrigo Morán, *Genetics*
Reinaldo Mueses, *FLAR**
Francisco Ortega, *Physiology*
Rosalba Reyes, *Genetics*
Francisco Rodríguez, *Genetics*
Humberto Rodríguez, *FLAR*
Luis H. Rosero, *Pathology*
Sory H. Sánchez, *Genetics*
Pedro Nel Vélez, *Genetics*
Jairo Vega, *FLAR*
Daniel Zambrano, *Physiology*

* *Left the project during 1997.*