

# Proceedings

of the  
*International Symposium on  
Rice Germplasm Evaluation  
and Enhancement*



*Organized by*

Dale Bumpers National Rice Research Center  
USDA-ARS and  
Rice Research and Extension Center  
Division of Agriculture - University of Arkansas

J.N. Rutger, J.F. Robinson, and R.H. Dilday, editors

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*J.N. Rutger, J.F. Robinson, and R.H. Dilday, editors*

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## **MISSION OF THE DALE BUMPERS NATIONAL RICE RESEARCH CENTER**

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### **ABSTRACT**

The mission of the Dale Bumpers National Rice Research Center, formerly known as the National Rice Germplasm Evaluation and Enhancement Center, is to conduct research directed at the needs of the rice industry: high yield, superior grain quality, pest resistance, and stress tolerance. Planning for the state-of-the-art Center began a decade ago, and construction was completed in 1998. The Center has 4,300 square meters of research laboratories, seed storage, greenhouses, and offices.

The Center will have eight USDA-ARS scientific groups: genetics, germplasm evaluation and enhancement, biology and control of weeds, cereal chemistry, molecular genetics, cytogenetics, molecular plant pathology, and plant physiology. The first four groups are staffed, a project leader is under recruitment for molecular genetics, and the final three project leader positions will be filled as funding permits. Shared laboratory space is also provided for University of Arkansas rice research groups, as well as visiting scientists.

Located on land leased from the University of Arkansas, the Center expands upon the long-time, cooperative federal-state rice research program at Stuttgart. The Arkansas Rice Research and Extension Center (RREC) was established in 1927, and the first USDA-ARS scientist at Stuttgart was employed in 1931. Staffing level remained at one to two USDA-ARS scientists until 1993, when the current expansion began.

The Center is a research center, and has considerable low temperature storage for research materials, but it is not the germplasm repository for rice. That function is being handled in the “working collection” of rice at the National Small Grains Collection in Aberdeen, Idaho, which curates, preserves, and distributes the U.S. rice germplasm collection, as it does the other small grains. In addition to the working collection at Aberdeen, the National Seed Storage Laboratory in Ft. Collins, Colorado, provides additional security by preserving a “base collection,” which intentionally duplicates materials in the working collection. Since rice cannot be grown outdoors at Aberdeen, the Stuttgart Center serves as the grow-out location for rice germplasm regeneration and increase, and then transfers these materials to Aberdeen.

### **INTRODUCTION**

The name of the former National Rice Germplasm Evaluation and Enhancement Center has been changed to the Dale Bumpers National Rice Research Center, hereafter referred to as the “Center.” The name change honors the dedicated efforts of the senior U.S. Senator from Arkansas, Dale Bumpers, in securing Congressional funding for construction and operation of this \$11.2 million state-of-the-art research facility. The name change also reflects the spectrum of research conducted at the Center. Enthusiastic and extensive support for the concept of a national Center has been provided by rice growers and industry leaders in Arkansas and other rice producing states.

The Center, a 4,300 square meter research facility consisting of laboratories, offices, and greenhouses, was completed in 1998. Planning and design for the Center began a decade ago through discussions amongst USDA-ARS, University of Arkansas, and rice industry leaders (R.H. Dilday, 1998a), and construction began in March 1996. Located on land leased from the University of Arkansas, the Center expands upon the long-time cooperative federal-state rice research program at Stuttgart. The RREC was established in 1927, and the first USDA scientist at Stuttgart, Dr. C. Roy Adair of the Division of Cereal Crops and Diseases, a predecessor agency of the Agricultural Research Service (ARS), was employed in 1931 (Adair et al., 1973). Staffing level remained at one to two USDA-ARS scientists until 1993, when current staffing expansion began. During all these years, from 1931 to the present, the University of Arkansas has provided research facilities for the ARS scientists. Thus, it is only fitting that laboratory space in the ARS Center be shared with UA RREC scientists. The combined group of eight ARS scientists and the seven UA RREC scientists will constitute a major concentration of rice scientists.

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## MISSION

The mission of the Center is to conduct research directed at the needs of the rice industry: high yield, superior grain quality, pest resistance, and stress tolerance. Research contributions of the Center will include development of new scientific knowledge on rice and its production practices, application of that knowledge to U.S. rice improvement, further evaluation of the U.S. rice collection and identification of “gaps” in the collection, and development of improved germplasm, genetic stocks, and gene pools for release to breeders and other interested rice scientists.

The mission is being accomplished through present or planned establishment of eight USDA-ARS research groups:

1. **Rice Genetics** - Determines inheritance of high yield, superior grain quality, and pest resistance; develops and releases improved germplasm and genetic stocks; and makes wide crosses to transfer disease resistance from other rice species (J. Neil Rutger, Director and Supervisory Research Geneticist, and Georgia C. Eizenga, Geneticist).

2. **Evaluation and Enhancement of Rice Germplasm** - Evaluates U.S. rice collection; expands collection of rice species; determines inheritance of allelopathy, pest resistance, and stress tolerance; and develops and releases diverse gene pools, genetic populations, and breeding stocks. (Robert H. Dilday, Research Geneticist, and Wengui Yan, Geneticist).

3. **Biology, Physiology, and Control of Weeds in Rice** - Identifies and exploits natural biological properties and mechanisms and discovers biological principals of economically important weeds, such as red rice and barnyardgrass, which will facilitate improved control of weeds. (David R. Gealy, Plant Physiologist, and Rebecca Chavez, Postdoctoral Research Associate).

4. **Cereal Chemistry** - Conducts research in rice grain chemistry, using the newest analytical techniques for identifying factors affecting grain quality in conventional and speciality rices, including aroma and taste. Undertakes a trait discovery program to identify added value grain traits for rice. (Rolfe Bryant, Research Chemist).

5. **Molecular Genetics** - Uses molecular marker technology to accelerate rice germplasm enhancement, collaborates with other laboratories to develop map-based cloning strategies, and uses genetic transformation to discover and introduce valuable genes for yield, grain quality, pest resistance, and stress tolerance. (Vacancy; under recruitment).

6. **Cytogenetics** - Transfers useful characters such as disease resistance, genes for high yield, and allelopathy from other rice species, and transfers apomixis through wide hybridization. (Planned as funding permits).

7. **Molecular Plant Pathology** - Identifies sources of disease resistance, especially for sheath blight, blast, and a new Fusarium-type disease, and accelerates incorporation of resistance genes into improved germplasm. (Planned as funding permits).

8. **Plant Physiology** - Determines factors needed to raise current yield levels, through investigations of grain-filling efficiency, growth and development, nitrogen use, and stress response. (Planned as funding permits).

The Center also provides shared laboratory facilities for University of Arkansas rice breeding, pathology, and plant physiology scientists.

As a national research facility, the Center interacts with scientists in international, national, and industry rice research agencies, especially in exchange of scientific information and germplasm. Some of the interactions will be achieved through hosting of meetings such as the present international symposium, and through a Rice Utilization Workshop being co-sponsored next March 10-12 with Elaine Champagne of the Southern Regional Research Center. Other interactions will be



achieved through “networking” meetings with USDA-ARS scientists conducting rice research in other locations: Beaumont, Texas; Davis, California; Albany, California; and New Orleans, Louisiana. And, of course, through the regular scientific meetings we all attend.

## **BACKGROUND OF THE U.S. RICE INDUSTRY**

### **Early Years**

On the world scene, the United States is a relative newcomer in rice production, having grown the crop slightly less than a mere four centuries. Rice arrived in the United States in the early 1600s, apparently brought by sailing ship from Madagascar (Dethoff, 1988). By 1609, rice was being grown in Virginia (Fiske, 1897, cited by Holder and Grant, 1979), and by 1686 was being cropped in South Carolina (Salley, 1919, cited by Holder and Grant, 1979). Rice was being grown in North Carolina by 1730, in Georgia by 1820, and in Florida, although South Carolina remained the principal producer until the U.S. Civil War (Holder and Grant, 1979).

After the Civil War, rice production began moving westward, first to Louisiana. By 1900, Louisiana was producing 69% of the total U.S. production of 197,000 tons, on a total of 146,000 hectares. Production soon expanded into Texas and Arkansas, which were producing 47% of the total U.S. crop of 1 million tons in 1909 (Holder and Grant, 1979). In subsequent decades, production expanded into its present major areas in the states of Arkansas, Louisiana, Mississippi, Missouri, and Texas, and California.

### **Current Production**

Current production of 8.3 million tons from 1.25 million hectares makes the average U.S. rice yields of 6.6 tons per hectare among the highest in the world. Yields have nearly tripled in the last 50 years while area in rice has about doubled (Fig. 1). All production is under flooded lowland conditions. Essentially all of the production is japonica rice, primarily temperate japonica in California and tropical japonica in the southern states. Some 72% of the production is long grain, virtually all from the southern region; 27% is medium grain and 1% is short grain. Almost all California rice is medium grain; Louisiana and Arkansas also have medium grains. The distribution of production by area is: Arkansas, 43%; California, 15%; Louisiana, 19%; Mississippi, 9%; Missouri, 4%; Texas, 10%; and a trace in Florida. About 40% of U.S. rice is exported.

### **Germplasm Introduction**

Among the early materials coming into the United States was a variety that became known as Carolina Gold, which was thought to have come in as mixture in Carolina White, a line introduced into South Carolina in 1694, presumably from Madagascar (Jones, 1937). Rutger (1992a, 1997) has observed both induced and spontaneous mutations for the recessive gene goldhull, *gh*, so it is likely that Carolina Gold arose by mutation.

Undoubtedly, other lines came into the United States in the early years, but modern day introduction dates from 1898, when Seaman A. Knapp was employed by the USDA as agricultural explorer (Adair et al., 1973). Knapp introduced 10 tons of Kiushu rice from Japan in the spring of 1899, for plantings in Louisiana and Texas—clearly quarantine regulations were not so rigid in those days.

The USDA began assigning Cereal Investigation (CI) numbers in 1903, apparently by a scientist in Louisiana (Anonymous, 1903). CI number 1, described as “Japan Upland,” was received from a firm in Richmond, Virginia. Assignment of CI numbers continued until the mid-1970s, when new lines began receiving Plant Introduction (PI) numbers. This change eliminated the confusion that sometimes arose because each of the cereal crops had separate CI systems, i.e., there was CI number 1 not only in rice, but also in wheat, barley, oats, and rye.

Many early rice CIs were lost; the lowest CI number in the current collection is CI 8, *Ostiglia*, received from a firm in Germany (Anonymous, 1903). The U.S. rice collection grew to over 4,400 entries by 1968 (Webb et al., 1985), and to over 17,000 in the active collection today (Bockelman, personal communication, 1998).

In the early 1980s the USDA’s Agricultural Research Service (USDA-ARS) encouraged and facilitated the establishment of Crop Advisory Committees (recently redesignated Crop Germplasm Committees) for about 40 crops or crop groups. The Rice Crop Advisory Committee was organized in 1983. From the beginning, the Rice Committee recognized the need for and recommended evaluation of the collection, and the USDA-ARS has funded a project on Rice Germplasm Evaluation and Enhancement at Stuttgart, Arkansas. The germplasm evaluation and enhancement project is discussed by Dilday (1998b; these proceedings).

## Research Beginnings

Rice research in USDA effectively began with Seaman A. Knapp's introduction of 10 tons of Kiushi in 1899 (Adair et al., 1973). From the early days, the USDA research programs were cooperative with state programs. Rice experiment stations were established in Louisiana and Texas in 1909, and California in 1912, and in Arkansas in 1927. Until the 1970s, the cooperative rice breeding efforts generally involved USDA-ARS breeders working in conjunction with other federal and state experiment station scientists, on state experiment stations. During the last three decades, state and/or industry breeders have assumed the lead in rice breeding, except in Texas, and USDA-ARS has phased its scientists into more basic fields such as genetics, rice grain quality, plant pathology, biology and control of weeds, and germplasm evaluation and enhancement.

## RESEARCH NEEDS

The eight projects in the Center will address one or more of the research needs of the rice industry: high yield, superior grain quality, pest resistance, and stress tolerance. Of course these needs are not unique to the United States; they are being addressed worldwide. Below are some of the research opportunities for the first need, high yield.

### High Yield Incremental Improvements

Incremental improvements in yield continue to be made through conventional pedigree breeding in the United States. New varieties often are developed from crosses between closely related elite germplasm lines, a situation occurring in other crops as well. Thus, Rasmusson and Phillips (1997) recently postulated that the continued plant breeding progress observed in malting barley, in what appears to be a narrow gene pool, is arising from newly generated variation. They hypothesize that genetic diversity is arising from *de novo* variation and elevated epistasis, as well as from the original diversity in the parent lines. Continued progress in the Illinois long-term selection studies for modified oil and protein in maize is a similar example; after more than 90 generations of selection, variability is still present (Dudley and Lambert, 1992).

Rather separate gene pools were in use in California vs. the southern United States for many years, with temperate japonicas in the former and tropical japonicas in the latter. In the last decade, these separations have begun to blur, with the release of several varieties from crosses between elite California varieties and elite southern varieties. In California, the long-grain varieties L-201, L-202, L-203, and L-204 have been produced in this fashion, and in the southern U.S. varieties produced in this way include Maybelle, Jackson, Rosemont, Adair, Alan, Millie, Cypress, and Litton (Anonymous, 1997).

Much of the crossing between related lines are within japonica semi-dwarfs, since semi-dwarfs now are grown in most U.S. rice growing areas. In Arkansas, improved high-yielding, relatively short stature varieties not carrying the semi-dwarfing gene, have continued to equal or exceed yields of semi-dwarfs, and generally have demonstrated better adaptation to the slightly rolling terrain of the Arkansas Grand Prairie, such as around Stuttgart. Semi-dwarfing in the United States was achieved in two ways: by crossing with the DGWG-TNI-IR8 tropical source followed by backcrossing to the local japonicas to recover satisfactory grain quality, and by induced mutation in locally adapted varieties. Induced mutation in the California variety Calrose produced the useful semi-dwarf Calrose 76, which has been shown to carry a semi-dwarfing gene, *sd 1*, at the same locus as the tropical source (Rutger et al., 1977). Calrose 76 and its progenies have been used in rice-breeding programs in California and other temperate rice areas, including Australia and Egypt. However, most present U.S. rice varieties received their semi-dwarfing gene from the tropical source, or from crosses between the tropical and induced mutant source, where the origin is no longer distinguishable.

### Hybrid Rice

In the private sector, the U.S. company RiceTec Inc. has an extensive hybrid rice development program (Stroike, 1996). In the United States there are twin hurdles that hybrid rice must clear: high cost of hybrid seeds, and suitable grain quality for U.S. markets. As in other parts of the world, U.S. rice geneticists are seeking improved genetic mechanisms for hybrid seed production, namely photo- or thermo-period sensitive genetic male sterility (2-line), and apomixis (1-line). For 2-line mechanisms, the author and associates have concentrated on selecting mutants for photoperiod sensitive genetic male sterility (Rutger and Schaeffer, 1994; Oard et al., 1991; Rutger, 1997), as has Mackill and associates in California (Han and Mackill, 1998). Further studies are underway.

Intensive searches for apomixis, or 1-line hybrids, are underway here as in Asia (Li and Rutger, 1998). Rutger et al. (1986) launched a search for apomixis in rice in California in 1985. Lack of success in finding apomixis in cultivated rice and in A-genome wild species entries of rice (Rutger, 1992b) has led the author to believe that molecular genetic techniques will be needed to either "turn on" apomixis in rice or to transfer genes from known apomictics.

### **Base Broadening with Indica Semi-dwarfs**

Since U.S. grain quality standards are so specific, breeders have been reluctant to go to non-elite materials and related rice species, unless no other source is available. However, the IRRI investigations on new rice ideotypes (Khush, 1993) have stimulated rice scientists worldwide to consider whether some new plant type might be desirable in their environments.

For the southern United States, the present author believes that indica semi-dwarfs are the improved plant type of the future. For well over a decade rice breeders in the southern United States have observed that conventional indica varieties from China yield considerably more than standard tropical japonica varieties (Bollich et al., 1988). These varieties include Gui-Chao, TeQuing, Cong-Gui and others, which appear to be “tall” semi-dwarfs. To further test the hypothesis that indica semi-dwarfs are higher yielding than tropical japonicas, McClung et al. (1998) conducted a two-year, multiple-location yield trial of seven recent U.S. varieties and seven indica varieties. The multiple location results are still being summarized, but a preview shows that the indica semi-dwarfs have a big advantage in Arkansas, where the top three indicas yielded 23% more than the top three japonicas, and were 11 days earlier in maturity (Rutger, 1998, unpublished). However, the indica varieties have grain quality that is unacceptable for U.S. markets: chalky grains, low head rice yields, and amylose contents 3 to 4% higher than southern long grains.

What makes the indica semi-dwarfs so high yielding? They grow aggressively, are more disease resistant and have more grains per panicle than the U.S. varieties. They universally have pubescent hulls and leaves, whereas most U.S. varieties now have glabrous hulls and leaves, but it does not seem likely that this single gene character affects yield. Efforts to recombine the high yield of the indica semi-dwarfs and the superior grain quality of the U.S. long grains have involved crossing and backcrossing to U.S. varieties followed by intense selection to recover the U.S. grain quality. Often the yield advantage has been eroded by the time satisfactory grain quality is recovered.

How can the high yield of the semi-dwarfs be recombined with U.S. grain quality? An alternative way might be to backcross in the opposite direction, i.e., to the semi-dwarfs, as was done in Korea some 25 years ago, when the traditional Korean japonicas were backcrossed in accelerated fashion to the semi-dwarf indicas from IRRI, resulting in the ‘Tongil’ types. The high-yielding Tongil types were widely grown for several years, however, preferences for short-grain quality appear to have led to a return to traditional japonicas (Anonymous, 1985).

Another possibility is to embark on a strategy of “Incorporation” or “base broadening,” as defined by Simmonds (1993):

“... the development of adapted populations from foreign stocks, en masse, the products being but distantly related by descent to the locally adapted genetic base. A population-oriented rather than a gene- or character-based approach is implied. Disregard of initial ill-adaptedness, of bad characters in general and determination to proceed slowly on a wide genetic base with material wholly unrelated to locally desirable stocks are necessary. Any such enterprise has to be bold (some would say foolish) and long-term in character.”

Simmonds cites as successful examples the reconstitution of high-yielding, disease-resistant potato and sugarcane germplasm using primitive, unadapted materials as starting points.

In my view, an opportunity for base broadening of U.S. rice is to assemble an array of high-yielding indica semi-dwarfs and redesign the grain quality of these semi-dwarfs to meet U.S. long grain quality standards. The redesign will be approached by intercrossing indica semi-dwarfs, which already are close to U.S. grain quality, followed by selection for U.S. grain quality within the progenies. Genetic engineering for grain quality factors may also be useful in such an effort. Candidates for inclusion in an indica semi-dwarf program include: the highly successfully IR 64, now grown on 11 million hectares (IRRI, 1985; Khush, 1997); newer Chinese semi-dwarfs such as Zhe 733 (Yan and Cai, 1991); and other recent indica semi-dwarf varieties from around the world. The F1's of an initial set of such indica/indica crosses involving the above varieties and additional breeding lines graciously provided by Dr. Gurdev S. Khush of IRRI are in the field at Stuttgart this season.

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## NATIONAL PLANT BREEDING STUDY

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The National Plant Breeding Study (NPBS) was undertaken in 1994 with funding provided by the Economics Research Service and the Cooperative States Research, Education, and Extension Service of the U.S. Department of Agriculture. To date, three reports have been published from the NPBS—

1. National plant breeding study—I  
Human and financial resources devoted to plant breeding in the United States in 1994 (Frey, 1996)
2. National plant breeding study—II  
National plan for promoting breeding programs for minor crops in the U.S. (Frey, 1997)
3. National plant breeding study—III  
National plan for genepool enrichment of U.S. crops (Frey, 1998)

In the first report, summaries were compiled on the basis of science person years (SY) and U.S. dollars. The total SYs devoted to plant breeding in the United States were 2241, with 67% of this number employed in the private plant breeding sector. SYs were summarized for 17 crop groups. The cereals group with 892 SYs accounted for 40% of the total plant breeding employment. Field corn accounted for a quarter of the total SYs with 545. The work in plant breeding was divided into three activities, plant breeding research, germplasm enhancement, and cultivar development. The proportions of plant breeding SYs devoted to these activities were 17% for plant breeding research, 18% for germplasm enhancement, and 65% for cultivar development. The financial input into U.S. plant breeding in 1994 was about \$550 million with 61% from the private sector.

In the second report, a national plan was constructed for assuring that breeding programs for minor crops will continue. It called for the establishment of a National Coalition for the Improvement and Use of Minor Crops (MCIC). The MCIC will be composed of persons from state agricultural experiment stations (SAES), Agricultural Research Service of the U.S. Department of Agriculture (ARS/USDA), private industry, and the agricultural and user communities who have expertise and familiarity with the problems in funding and sustaining research and development programs for minor crops. The coalition will be organized and administered by the Experiment Station Committee on Organization and Policy (ESCOMP). The mission of the MCIC is to organize a plan to sustain, and when deemed desirable, to expand breeding projects on minor crops a) to sustain environmental quality, b) to assure a continual, affordable, high-quality supply of diverse crop products for society, and c) to provide ready sources of raw materials for industry.

It is the third NPBS report entitled “A National Plan for Gene Pool Enrichment of U.S. Crops” that is the major focus of this paper (see Frey, 1998). Crop gene pool enrichment is defined as “the transfer of useful genes from unadapted lines of the same species, related species, and genera, or any plant, animal, or microbe species, by sexual or asexual technology (including transformation) into plant populations that are useful for developing new crop cultivars.”

The vision for the National Gene Pool Enrichment Plan is that crop gene pools with new and useful gene combinations will be made available to the U.S. plant breeding community. And the plan’s goals are: a) to develop a national plan and strategy for crop gene pool enrichment, including necessary human, physical, and fiscal resources, and b) to identify, access, discover, transfer, and make available genes critical to plant breeding objectives.

Crop gene pool enrichment in the United States generally has been sporadic and done without national systematic planning. Gene pool enrichment has been done quite extensively for tomato, potato, wheat, sorghum, maize, and cotton and introgressed genes have been released in new cultivars for these species. Gene pool enrichment has been less extensive for oat and soybean (see appendix of Frey, 1998).

The United States most systematic evaluation and gene pool enrichment program is currently being conducted on maize and is referred to as the Latin American Maize Project/Germplasm Enhancement of Maize (LAMP/GEM) Project. This project, led by ARS/USDA, includes partial funding by the private plant breeding sector, and is international in scope.

It encompasses the transfer of genes for both qualitative and quantitative traits. The donor maize lines are all *Zea mays* accessions, so gene pool enrichment is done in the traditional manner.

More than 20 genes for disease and insect resistances have been transferred from wild *Lycopersicon* species and used in cultivars of tomato. And recently, genes have been transferred to tomato for total fruit solids and salinity tolerance, which are quantitative traits.

The development of techniques for transformation in plants has brought a flurry of activity to transfer genes into plants from unrelated sources. The most highly publicized case of transforming plants is with *Bt* genes that give resistance to insects. Cultivars of potato, maize, and cotton with *Bt* genes are being grown on several million acres in the United States. The primary target insects are the European corn borer with maize, several boll worm species with cotton, and the Colorado potato beetle with potatoes. The ability to transform crop plants with genes from almost any living organism revolutionizes gene pool enrichment possibilities. For one thing, this ability to transform crop plants has brought the private sector into gene pool enrichment in an intensive but as yet narrowly focused way in relation to the genetic resources that are available. Transformation technology also permits the use of genes from very remote sources to customize plant products. New technologies now emerging will provide knowledge about the utility of specific areas of the genome. This knowledge, along with other technologies, may enable specific genetic traits to be efficiently recombined into new cultivars.

Plan of action for a national gene pool enrichment program has several aspects--

**1. Organize a Committee/Task Force/Coalition to Assess Needs and Promote and Coordinate Research Activities for Crop Gene Pool Enrichment**—This group would be organized and coordinated by the Cooperative States Research, Education, and Extension Service (CSREES/USDA) with help from ESCOP and it would be called the Crop Gene Pool Enrichment Coalition (CGEC). Given that most gene pool enrichment work, under this national plan, will be done in SAES, it is natural for CSREES/USDA, which provides partial funding to and liaison with SAES, to be the coordinator for the CGEC. The CGEC would be composed of persons from CSREES/USDA, ARS/USDA, SAES, and private industry who have expertise and familiarity with problems of organizing, funding, and sustaining gene pool enrichment programs for U.S. crops. **The mission of the CGEC is to develop and implement a national plan for the timely and orderly enrichment of the gene pools of U.S. crops to assure a continuous flow of cultivars that will (a) enhance environmental quality, (b) provide an affordable and high-quality supply of diverse crop products for society, and (c) assure ready sources of raw materials for industry and export demands.**

**2. Prioritize U.S. Crops According to Their Needs for Gene Pool Enrichment**—On the basis of databases developed by Crop Germplasm Committees (CGC) and potential CGCs, the CGEC will prioritize the needs of U.S. crops for gene pool enrichment.

**3. Fund Gene Pool Enrichment for U.S. Crops**—Because national food security, the world's food supply, food safety and nutrition, sustainable agricultural production, and competitiveness in world trade are highly dependent on the continual evolution of improved cultivars from the U.S. plant breeding effort, it is proposed that the National Plan for Gene Pool Enrichment of U.S. Crops be supported by a federally funded initiative of \$50 million annually. These funds will be used in the following way—

- a. Five million dollars to augment the National Plant Germplasm System (NPGS) to assure that needed plant genetic resources are available, evaluated, and distributed.
- b. Twenty-five million dollars for a competitive grants program for five-year projects to be administered by the National Research Initiative. Multidisciplinary research, international collaboration, and training would be emphasized. This program would be based upon the use of basic science in developing gene pool enrichment practices and programs.
- c. Twenty million dollars for grants to be administered by CSREES/USDA, awarded competitively according to the priorities of needs of crops for gene pool enrichment. Awards would be made on the basis of relevance and excellence and would emphasize multidisciplinary research, international collaboration, and training. This portion of the program will mandate that collectively, over all projects, that federal support is to be matched by equal support from SAES and from the private sector, thus developing \$60 million annually for the gene pool enrichment program.

**4. Build a Consortium to Support the Agenda of CGEC**—Gene pool enrichment for U.S. crops is critical because the resulting plant populations are the raw materials from which cultivars will be developed one to three decades from now. Every U.S. citizen has an absolute and immediate dependence upon a quality environment and assured food supply at reasonable cost. Plant breeding is center stage to assure that these dependencies are met via genetic solutions.

a) The CGEC will involve public institutions and private companies that do plant breeding, non-governmental organizations, farmers, and end-user groups in educational programs and consortia to build broad-based public support for the crop genepool enrichment program. The CGEC will provide leadership, develop educational materials, and organize the crop genepool enrichment project into a public awareness program.

b) The CGEC will organize a “crop gene pool enrichment” committee with representation from various groups to aid this effort in education programs, informing governmental agencies, and public awareness programs.

**5. Develop Models for Distribution of Enriched Gene Pools**—As a general principle, crop gene pools, enriched in projects supported entirely or largely by public funds, should be distributed freely to collaborative breeders in the United States. Some gene pool enrichment will be done by private industry, and more specifically, by an individual company—certainly, these cases will be subject to strict intellectual property rights protection. A major task for the CGEC will be to provide consensus models for release and use of enriched gene pools that can be modified to meet specific needs.

**6. Develop Human Resources**—The CGEC will develop short courses for training plant scientists on traditional and unique technologies for crop gene pool enrichment. Specialty workshops will be developed for training on new techniques as they are developed.

**7. Evaluate the National Crop Gene Pool Program Impact and Performance**—Responsibility for assigning gene pool enrichment projects for individual crops will be done on the basis of competitive proposals for initial periods of five years. One year before the initial grant period ends, the CGEC will assign a committee to evaluate a project’s progress. This evaluation will be used to judge whether the project should be continued, modified, moved, or have other disposition.

At five-year intervals, the national crop genepool enrichment program will be evaluated by a task force made up of scientists and administrators who are not members of the CGEC. This task force will evaluate (a) whether the CGEC is focusing on the vision statement and its mission, (b) administrative effectiveness, (c) general progress of the national program, and (d) effectiveness of educational, human resource, and funding programs.

## SUMMARY

Keeping U.S. agriculture strong, sustainable, and competitive will require a national effort for the enrichment of U.S. crop genepools from which improved cultivars can be developed 10 to 30 years hence. A National Gene Pool Enrichment Program for U.S. crops has been proposed. This program would be guided by the Crop Genepool Enrichment Coalition (CGEC) composed of persons from SAES, USDA, and private industry and administered by the CSREES/USDA with help from ESCOP. The mission of CGEC is to develop and implement a national plan for the timely and orderly enrichment of the gene pools of U.S. crops to assure a continuous flow of cultivars that will (a) enhance environmental quality, (b) provide an affordable and high quality supply of diverse crop products for society, and (c) assure ready sources of raw materials for industry and export demands. Foundation funding for crop gene pool enrichment would be an annual \$50 million appropriation from the U.S. government to be administered by CSREES/USDA.

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## RICE GERmplasm EVALUATION AND ENHANCEMENT AT THE DALE BUMPERS NATIONAL RICE RESEARCH CENTER

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### ABSTRACT

The USDA-ARS rice germplasm working collection contains 17,279 accessions from 110 countries or regions. The Dale Bumpers National Rice Research Center (DB NRRC) at Stuttgart, Arkansas coordinates the systematic evaluation of the U.S. rice germplasm collection. Data for more than 30 different descriptors have been collected and are part of the germplasm resources information network (GRIN). Some of the descriptors include allelopathy, amylose content, days from emergence to flowering, disease resistance, grain type, herbicide tolerance, kernel length, kernel weight, lodging, plant height, salt tolerance, yield, etc. A broad range of genetic variability is present in GRIN for each descriptor. For example, amylose content ranges from 0.0 to 53.0%, days from emergence to flowering (37 to 219 days), kernel length (3.0 to 9.9 mm), kernel length/width ratio (1.0 to 8.0), plant height (41 to 208 cm), protein content (1.7 to 13.6%), salt tolerance rating (0.0 to 5.6), straighthead rating (3.0 to 9.0) and 1,000 kernel weight (6.9 to 46.0 g). Some accessions are tolerant to nonselective herbicides such as glyphosate and sulfosate, other accessions significantly increase crayfish production while other accessions apparently are allelopathic to weed species such as duck salad (*Heteranthera limosa*) and barnyardgrass (*Echinochloa crus-galli*). High-performance liquid chromatography (HPLC) chromatograms show significant differences in 5 different substances (peaks) when allelopathic accessions (i.e., PI 312777) are compared to accessions that demonstrate little or no allelopathic activity (i.e., Rexmont). Although the USDA-ARS rice germplasm collection is very diverse, there are 'gaps' in the collection. For example, only 10 countries constitute 81% of the collection. Currently, germplasm is being introduced into the United States from Bangladesh, China, Colombia, Hungary, India, Japan, Korea, and Taiwan. Germplasm from these countries has demonstrated apparent allelopathy to weed species, disease resistance, earliness, herbicide tolerance, high yield potential, and superior grain quality.

### INTRODUCTION

The rice portion of the USDA-ARS National Small Grains Collection (NSGC) contains 17,279 accessions from 110 countries. Prior to 1988 the collection had not been systematically evaluated; consequently, it had not been efficiently used in varietal development programs. For example, in 1990 only 56 genotypes, which can be traced to 13 accessions, had been used in developing cultivars in Arkansas, 49 genotypes traced to 12 accessions had been used in developing cultivars in Texas, 49 genotypes traced to 16 accessions had been used in developing cultivars in Louisiana, and 57 genotypes traced to 23 accessions had been used in developing cultivars in California. Furthermore, 10 of the 12 and 13 parental accessions in the Texas and Arkansas breeding programs, respectively, were identical and 8 of the 13 and 16 accessions in the Arkansas and Louisiana breeding programs, respectively, were identical (Dilday, 1990). Since 1988, a systematic evaluation of the collection has been coordinated by the USDA-ARS rice germplasm project at Stuttgart. Important characters such as weed suppression (allelopathy), herbicide tolerant and stress tolerant germplasm have been identified. A thorough and systematic evaluation and characterization of the collection is on-going and the promising germplasm is being enhanced so it can be used in varietal development programs.

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There are seven management activities (acquisition, preservation [rejuvenation], distribution, description [evaluation], enhancement [prebreeding], breeding, and biotechnology) of ARS's germplasm plan (Anonymous, 1991; Chelimsky, 1990). A key objective of the National Plant Germplasm Systems Long-Range Plan is the acquisition of new germplasm and evaluation of existing germplasm collections (USDA, 1981). The Implementation Plan states that "there are two principal reasons for evaluating germplasm in the USDA-ARS collections: (a) to identify "gaps" in the range of genetic diversity that might be filled through acquisition efforts; and (b) to enhance the usability of the collection through a more intelligent response capability as users identify their specific needs. The ultimate objective is to have germplasm used in crop-improvement research and development. For germplasm to be used, it must be shown to be useful. Evaluation identifies useful attributes. These attributes include those traits (phenotypic expressions) that are of interest to the developer of the crop such as environmental stresses and biotic stresses (pests); quality traits; yield and yield constraint traits. Furthermore, a major objective of the ARS six-year Implementation Plan (1992-1998) is to develop means for maintaining and increasing the productivity and quality of crop plants (Anonymous, 1991). A component of this objective is to broaden the germplasm resources of plants to ensure maximum genetic diversity for improved productivity through collection, classification, evaluation, preservation, and distribution of plant germplasm and assess its potential for meeting agricultural needs. Although more than 300,000 individual rice accession characters have been evaluated since 1988, the USDA-ARS rice germplasm collection remains insufficiently evaluated and consequently insufficiently enhanced for key traits. This has resulted in insufficient use of suitable genetic diversity.

The USDA-ARS rice germplasm project at Stuttgart coordinates the national evaluation of the USDA-ARS rice germplasm collection and the principal objectives focus on areas, which are not adequately investigated by other public institutions or private industry; although, the project is cooperative with state institutions and private industry and complements their ultimate goal of rice variety improvement. In addition, this project complements the objectives and mission of the USDA-ARS National Small Grains and Potato Research Unit at Aberdeen, Idaho and the USDA-ARS National Small Grains Laboratory at Fort Collins, Colorado.

Current technology for rice germplasm evaluation, enhancement, and genetic analysis varies with each agronomic characteristic. More than 30 plant characteristics have been evaluated for many of the 17,279 accessions and these data are currently in the USDA-ARS Germplasm Resources Information Network (GRIN). The rice germplasm project at Stuttgart collected and supplied much of the data into GRIN. Current technology will be available at the recently completed USDA-ARS DB NRRC. Current technology for evaluation, enhancement, and genetic analysis will range from standard conventional methods to more recent advances in gene manipulation through isozyme analysis, southern and northern blotting methods for detecting and analyzing DNA/RNA, and measuring genetic variation by restriction fragment length polymorphism (RFLP). The development and use of different gene maps (genetic, RFLP, physical, restriction, and DNA/RNA) are described in the USDA-ARS Plant Genome Research Program (Miksche, 1994).

Significant progress on the evaluation, enhancement, and genetic analysis of the USDA-ARS rice germplasm collection will impact essentially all aspects of rice research, development, and production. For example, from 1990-1994 Arkansas had about 43% of the rice acreage and produced about 41% of the rice in the United States. The estimated value of the rice crop in Arkansas in 1995-96 was about \$550 million annually (Anonymous, 1997). However, in Arkansas alone annual losses due to red rice were estimated at \$13.0 million, losses due to diseases were estimated at 10% of the total value of the crop (\$55.1 million), herbicide cost was estimated at \$45.00/acre (\$58.5 million), fertilizer cost at \$40.00/acre (\$52.0 million), and water cost at \$35.00/acre (\$45.5 million). Furthermore, inadequate stand establishment has been estimated to cost the rice producer an additional \$25.00-\$50.00/acre (\$32.5-\$65.0 million) (Slaton et al., 1994).

The newly identified germplasm has demonstrated resistance to certain environmentally safe herbicides (sulfosate, glyphosate etc.) for red rice control and weed suppression or allelopathic activity for weed control. Drought and salt tolerant lines with better water use efficiency and germplasm with the ability to germinate through both soil and water for better stand establishment are being introduced into the collection. Also, rice germplasm has demonstrated its diversity in the use of nitrogen fertilizer and disease and insect resistance. Savings to the rice producers throughout the southern states of Arkansas, Louisiana, Mississippi, Missouri, and Texas and the western state of California theoretically will amount to millions of dollars annually as the new germplasm is incorporated into cultivars.

Research results developed by this project are being used by other federal, state, and industry scientists; scientists involved in rice research internationally, and extension personnel. For the new and exotic germplasm to be used effectively there must be a cooperative efforts between geneticists, plant breeders, plant physiologists, plant pathologists, weed scientists, entomologists, molecular biologists, soil scientists, chemists, and food technologists. The ultimate user of the product will be the rice producers in the form of new and improved cultivars. The ultimate beneficiary will be the American public.

The research can ultimately lead to less use of water through the development of germplasm with greater water use efficiency. Also, the development of germplasm that requires less use of herbicides or the use of environmentally safe

herbicides, fungicides, and nitrogen fertilizer will result in better water quality and less environmental contamination. Also, the research can lead to less use of water through the development of germplasm with greater water use efficiency. Newly developed or enhanced germplasm is the product of this research and the germplasm has been incorporated into conventional breeding programs.

## PROCEDURES

Rice germplasm research conducted or coordinated at DB NRRC will emphasize four approaches: (1) evaluation, (2) enhancement/genetic analysis, (3) characterization, and (4) introduction of additional *Oryza* species.

(1) Evaluation — Field and laboratory evaluation experiments for useful characters are conducted or coordinated through the germplasm project at DB NRRC. Emphasis will be on evaluating for: (a) allelopathy, (Dilday et al., 1994) (b) herbicide, salt and drought tolerance or increased water use efficiency, (d) disease and insect resistance, (Bernhardt, 1994; Lee, 1992) (e) milling quality, (f) nitrogen efficiency, (Wells et al., 1973; and Norman et al, 1995) (g) seedling vigor, and (h) ratooning ability. Plant extracts will be evaluated in laboratory, greenhouse, and growth chamber experiments. Root and shoot exudates and soil extracts will be evaluated by methods such as the continuous root exudate trapping system (CRETS), incorporation of plant material into growth media in vitro and in vivo and soil related experiments. Product identification will be conducted by isozyme analysis, HPLC, gas chromatography (GC) and paper and thin-layer chromatography. Plant behavior studies involving drought tolerant germplasm will be conducted. The root pulling resistance (RPR) method and photosynthetic systems that monitor photosynthetic rate, stomatal conductance, respiration, and transpiration will be used. Ultimately the products will be field evaluated to determine their potential in controlling weed species, manipulating plant growth development and monitoring plant responses to drought, salt, field and milling yield, etc. The research will be conducted independently and in cooperation with other scientists.

(2) Enhancement/genetic analysis — Some of the new and exotic rice germplasm that is presently being identified or developed in this project is in feral backgrounds. This germplasm will be enhanced so it can be used in U.S. rice breeding programs. Some approaches that can be used to enhance the germplasm may range from traditional methods such as hybridization and selection to procedures such as electroporation (Fromm et al., 1985), particle bombardment (Klein et al., 1987), regenerable callus (Oard and Rutger, 1989), callus induction (Oard and Rutger, 1989), plant regeneration from protoplast (Abdullah et al., 1986; Kanda et al., 1988; Kyojuka et al., 1987) and anther culture (Mirlohi et al., 1989). The latter methods will be conducted in cooperation with scientists with expertise in biotechnology, which includes graduate students at the University of Arkansas and other research laboratories.

(3) Characterization — Phenotypic plant and seed characteristics of each accession will be described and catalogued in GRIN for use by national and international scientists. In addition, genotypic differences and similarities will be characterized and catalogued. These data along with the evaluation and passport data for each accession will serve as the database in developing strategies for a core collection of rice germplasm.

(4) Germplasm introductions — There are about 81,000 accessions of rice at the International Rice Research Institute (IRRI) in the Philippines. China has several rice germplasm collections but the collection at Beijing has over 50,000 accessions. The national rice collection in India has over 35,000 accessions and is located at several storage sites. The Japanese national rice collection contains about 26,000 accessions. The USDA-ARS rice collection has 17,279 accessions from 110 countries. The IRRI collection is probably the most genetically diverse rice collection in the world because the acquisition and field collection efforts were implemented in the appropriate places and at opportune time before advanced genetic erosion occurred (Chang et al., 1989). However, it has been estimated that a total of 100,000 rice cultivars exist in Asia alone (Chang, 1985). Therefore, the collection and acquisition of rice germplasm must be a continuous effort. Moreover, the important point is that only a small proportion of the total genetic diversity of rice has been conserved (Chang et al., 1989).

Gaps in three important areas of the USDA-ARS rice germplasm collection exist. These three areas include germplasm introductions from (1) the IRRI germplasm collection, (2) India, Russia, Colombia, Brazil, Japan, China, and other Asian and European germplasm collections, and (3) insufficient accessions of different *Oryza* species. The first two areas should be addressed independently and immediately because access to some of these collections is greater now than it has been for decades. For example, the rice germplasm collection at IRRI is available and at one location; whereas, the other germplasm collections are not as accessible and sometimes are not at a central location. Some IRRI accessions have been received at

the USDA, ARS National Seed Storage Laboratory (NSSL) at Fort Collins, Colorado. However, a judicious approach must be used in introducing germplasm into the United States because some foreign collections contain groups of breeding lines and these accessions do not increase the genetic diversity of a collection. However, rice accessions throughout the world should be incorporated into the U.S. rice collection, either at NSSL and/or NSGC, when the world political environment permits their acquisition and introduction because many of the world germplasm banks can be closed at any time.

The exact number of *Oryza* species is controversial. However, the genus *Oryza* is generally thought to include between 20 to 24 species. Approximately 15 species have the same diploid number ( $2n=24$ ) as *O. sativa*; whereas, nine species are tetraploid ( $2n=48$ ). Prior to 1995, seeds of only five *Oryza* species were available for distribution from the rice portion of the National Small Grains Working Collection that is housed at Aberdeen, Idaho. However, the major portion of the collection is comprised of *Oryza sativa*, the genus and species of cultivated rice in the United States.

## RESULTS AND DISCUSSION

**HISTORY:** Rice germplasm acquisition, rejuvenation, storage, distribution, evaluation, and enhancement activities are part of the U.S. National Plant Germplasm System (NPGS). NPGS is coordinated by the USDA-ARS. Therefore, final decisions on rice germplasm are made by the ARS Administrator, Floyd Horn, the Assistant Administrator for Germplasm, Henry Shands, and the National Program Leader for Small Grains, Charles Murphy. Although the NPGS is coordinated by ARS it is composed of scientists from the federal, state, and private industry.

The Seed and Plant Introduction Office was organized in 1897. It was organized to: 1) acquire and maintain germplasm, 2) preclude the introduction of new diseases, insects, and weeds; and 3) distribute introductions to scientists. Accessions are identified by either Cereal Investigation (CI) numbers or Plant Introduction (PI) numbers. The CI numbers are consecutive numbers that are assigned within an individual crop. Conversely, PI numbers are consecutive numbers that are assigned across all crops. Therefore, the same CI number can be assigned to several accessions in several crops. For example, CI 8 in rice (*Oryza sativa* L.) is "Ostiglia" and CI 8 in Oat (*Avena sativa* L.) is "Gray Winter," however, PI numbers are consecutive and unique numbers. The first rice germplasm accession which was introduced into the United States and still remains in the rice working collection at Aberdeen, Idaho is Ostiglia (CI 8). Ostiglia was introduced from Germany in 1904 by Haage and Schmidt. Official germplasm collections in the United States did not exist until Congress passed the Research and Marketing Act of 1946. The Research and Marketing Act of 1946 was a special appropriation whereby Congress appropriated funds for 1) quarantine nurseries, 2) maintenance of viable seed, 3) accumulation of data, 4) cataloging information 5) filling seed request, and 6) grouping all of the small grains into one collection. Consequently, the National Small Grains Collection (NSGC) was organized as an official project in 1948 (Wesenberg et al., 1992).

Prior to 1948, germplasm accessions were collected by various individuals and researchers or scientists maintained their own germplasm collections. There was no central, organized germplasm program. For example, individual rice germplasm collections were maintained at Stuttgart, Arkansas; Biggs, California; Crowley, Louisiana; and Beaumont, Texas. However, after 1948 many of the individual germplasm collections became part of the NSGC. One example is the rice collection maintained at Stuttgart by C. Roy Adair. Roy Adair was the first USDA-ARS scientist located at the Rice Research and Extension Center, Stuttgart (1931-1952). The rice collection at Stuttgart officially began in 1932 when a local farmer, H.D. Dilday, presented the first rice accession to Roy Adair at the 1932 rice field day. H.D. Dilday was one of the local farmers that collected money in 1925 to purchase the 160 A where the DB NRRC is located. The land was purchased in 1925 and donated to the University of Arkansas and became the Rice Research and Extension Center at Stuttgart in 1927. Also, H.D. Dilday was a charter member of the Arkansas Rice Growers Association (Riceland Foods), charter member of the Arkansas Farm Bureau, and a charter member and the first vice president of the Stuttgart Cooperative Buyers Association. Because of his involvement in the rice industry, H.D. Dilday along with another local farmer, W.N. Beeble, and the President of Peoples National Bank (Stuttgart), Paul R. McCoy, served as the three-member Visitors Committee at the first Rice Research and Extension Center Field Day, 13 April 1932. Roy Adair left Stuttgart in 1952 to become Investigation Leader of the USDA-ARS Cereal Crops Research Branch, Beltsville, Maryland. Most of Roy Adair's rice germplasm collection at Stuttgart became part of the USDA-ARS rice germplasm collection, which has grown to 17,279 accessions from 110 countries.

**COLLECTION AND PRESERVATION:** Although the USDA-ARS rice germplasm collection has grown to 17,279 accessions from 110 countries there are obvious gaps in the collection. For example, five countries make up 60% of the collection, 10 countries make up 81% of the collection, and 78% of the collection was introduced prior to 1980. Central and South America, the continent of Africa, Russia, India, and Asia are areas where rice germplasm must be acquired to fill obvious gaps. Furthermore, 97% of the rice germplasm from Bangladesh, 96% from China, 94% from Hungary, 92% from Indonesia, and 94% from Japan were introduced into the USDA-ARS rice germplasm collection before 1980. There have been 1,843 accessions from Indonesia, 1,543 from Colombia (CIAT) 1,041 from Bangladesh, 451 from Japan, and 204

from China introduced into the NPGS since 1993. Rice germplasm that is in the NPGS is preserved at two locations in the United States. The base collection is maintained at Fort Collins, Colorado, and the working collection is maintained at Aberdeen, Idaho.

**REJUVENATION AND EVALUATION:** When accessions in the USDA-ARS rice working collection drops below 50g then the accession is increased (rejuvenated) at DB NRRC and returned to the NSGC. Also, after a rice accession has cleared quarantine initially and before it is introduced into the NPGS, the accession is increased at DB NRRC and sent to the NSGC at Aberdeen, Idaho. From 1993 to 1998, there were 5,217 low inventory accessions and 2,057 accessions that had cleared quarantine that were rejuvenated at DB NRRC. The following characteristics have been evaluated and entered into GRIN with the range of diversity in parenthesis: alkali/spreading value (2.00 to 7.04); allelopathy (387 accessions); amylose percent of milled rice (9.00 to 54.00), aromatic rice (130 accessions); awning-absent (7,710), short and partly awned (3,307), short and fully awned (68), long and partly awned (596), long and fully awned (826); blast-IB1, IB33, IB49, IC17, IE1K, IG1, and IH1; sheath blight (3.0 to 9.0); bran color-white (750), light brown (14,033), speckled brown (44), brown (465), red (2942), variable purple (3) and purple (49); days to anthesis (coded value); days from emergence to flowering-37 to 59 days (90), 60 to 81 days (1,072), 82 to 104 days (5,965), 105 to 126 days (4,101), 127 to 148 days (1,075), 149 to 171 (391), 172 to 193 (87), 194 to 216 (1) and 217 to 238 (1); endosperm type-common or non-waxy (6,897), glutinous or waxy (300); gelatinization temperature-high (103), intermediate (3,339), low (3,804); grain type- short grain (2,585), medium grain (6,562), long grain (4,497), extra long grain (196), other (8) and mixed grain lengths (242); hull color-white (75), straw (10,422), gold, light or deep (1,217), tawny or russet (281), furrowed- gold, dark or purple (613), mottled, speckled or piebald (594) purple (248), black (105), mixed or intermediate (519); hull cover-glabrous (1,752), hairs on lemma keel only (121), hairs on upper portion only (185), short hairs throughout (11,570), long hairs throughout (415), hairs on both lemma and palea keels (3) and mixed types (41); kernel length (3 to 10 mm); kernel weight in milligrams of milled rice (7.70 to 29.57); length/width ratio for brown dehulled rice (1.00 to 5.76); kernel width in millimeters for brown dehulled rice (1.00 to 4.01); kernel weight of 1,000 kernels (3.37 to 46.93 g); lodging-0 to 10% (1,764), 11 to 20% (1,260), 21 to 30% (3,609), 31 to 40% (1,384), 41 to 50% (2,553), 51 to 60% (586), 61 to 70% (1,554), 71 to 80% (944) and 81 to 100% (1,581); panicle type- compact (401), intermediate (13,496) and open (1,220); parboil loss based on the percentage of solids lost when parboiled (7.00 to 58.03); plant height (coded values) in cm- less than 70 (349), 80 to 89 (1,098), 90 to 99 (1,565), 100 to 109 (1,606), 110 to 119 (1,654), 120 to 129 (1,915), 130 to 139 (1,839), 140 to 149 (1,274) 150 to 159 (674) and 160 or more (446); plant height measured in actual values (20 to 209 cm); plant type- erect (2,043), intermediate (4,787), open (7,561), spreading (798), and procumbent (8); protein % (1.70 to 14.57); ratooning-no growth (2,286), vegetative growth (3,521), head formed but no mature seed (1,544) and mature seed (726); tolerance to 1.0 lb a.i. of glyphosate and sulfosate (16); salt tolerant- 0=sensitive and 10=tolerant, 0.00 to 0.80 (12,636), 0.81 to 1.62 (1,051), 1.63 to 2.43 (242), 2.44 to 3.24 (103), 3.25 to 4.05 (26), 4.06 to 4.86 (8) and 4.87 to 5.67 (4); sterile lemma color- straw (9,671), gold (236) red (91) and purple (217); straighthead- no symptoms (5), less than 1.0% malformed panicles (14), 5% malformed panicles (58), 10% malformed panicles (239), 30% malformed panicles (725), 50% malformed panicles (1,089), 70% malformed panicles (1,329), 100% malformed panicles (929) and severe damaged with plants not heading (252); and upland type (331). Additional data can be obtained from the internet <[www.ars-grin.gov](http://www.ars-grin.gov)>.

**ENHANCEMENT:** Enhancement activities at the DB NRRC include allelopathy or weed suppression to both ducksalad and barnyardgrass; disease resistance for blast, sheath blight, and straighthead; stress tolerance for drought, herbicides, and salinity; earliness; quality and high yields.

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# MANAGING THE WORLD'S LARGEST COLLECTION OF RICE GENETIC RESOURCES

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## ABSTRACT

The International Rice Genebank at the International Rice Research Institute (IRRI) holds in trust the world's largest, and probably most diverse collection of rice genetic resources. There are over 84,200 accessions in the collection, and more than 18,000 samples for registration. Most accessions are Asian cultivated rice varieties (*Oryza sativa*), but there are significant collections of *O. glaberrima*, the West African cultigen, and all known wild *Oryza* species.

Since 1991, significant changes have been made to genebank operations for germplasm multiplication and regeneration, seed handling and conservation, data management, and research has expanded on the applications of biosystematics to facilitate conservation and use of the wild rices. We recently began molecular marker studies to understand the diversity of wild and cultivated rices, as well as identify duplicate accessions.

The international policy environment for conservation and use of germplasm has changed since the coming into force of the Convention on Biological Diversity (CBD) in December 1993. Furthermore, the Agreement signed with Food and Agricultural Organization (FAO) in October 1994 has placed additional responsibilities on IRRI for the management of this important germplasm collection. The use of Material Transfer Agreements is just one of the consequences of the new germplasm regime.

Since 1994, IRRI has worked with almost 30 countries to collect cultivated and wild rices, supported by the Swiss Agency for Development and Cooperation (SDC). Data management staff have worked with germplasm personnel in five countries to develop PC-based rice information systems that are closely based on the International Rice Genebank Collection Information System (IRGCIS).

The genebank meets all the international standards for *ex situ* conservation of seeds that were approved by the FAO Commission on Genetic Resources for Food and Agriculture, since the renovation of the genetic conservation infrastructure at the Institute and introduction of revised protocols for germplasm management. These protocols were based on research about seed quality, viability, and production environment. Matching varieties and growing season has led to significant increases in seed quality (both in terms of viability and seed health).

Recent applications of molecular markers are beginning to unlock the diversity of wild and cultivated rices. The demonstration of genome homoeology across grass genomes offers exciting opportunities to exploit genetic information in rice and other cereals to search for traits of economic value in this most important of food crops.

## INTRODUCTION

The IRRI was founded in 1960, and from the early days of the Institute, conservation, evaluation and use of rice genetic resources have been important activities of its research agenda. The early impact of the high-yielding rice varieties for example was due to the exploitation of genetic diversity for dwarf stature in the Chinese landrace, Dee-geo-woo-gen.

For almost 30 years, the eminent rice geneticist, Dr. T. T. Chang, led the genetic resources activities at IRRI. Under his leadership, the collection in the International Rice Genebank (formerly the International Rice Germplasm Center) grew to more than 80,000 samples. Since 1991, the aim has been to consolidate the progress over the previous three decades (Chang 1985), while taking the opportunity of new technological developments, research findings, and policy environment to significantly upgrade the operations and procedures of the genebank. The International Rice Genebank is widely recognized as conserving one of the world's most important genetic resources collections, which is maintained to international standards (SGRP, 1996; 1997).

### **The Scope of the International Rice Genebank Collection (IRGC)**

The collection in the International Rice Genebank currently comprises more than 102,700 samples. Of these, more than 84,200 are registered accessions, and a further 18,500 samples have been received in the past three years, and are undergo-

ing seed multiplication prior to being assigned an accession number in the collection. The IRGC includes not only landrace varieties and wild species, but also current and obsolete modern varieties, advanced breeding lines, and genetic stocks. Asian rice varieties (*Oryza sativa*) comprise the bulk of the collection, with almost 80,000 accessions. There are more than 1,200 accessions of *O. glaberrima* landraces from West Africa. All the wild species of *Oryza* are included in the more than 3,000 accessions in the collection (Table 1). *O. schlechteri* from Papua New Guinea is the rarest species in the collection, represented by just one vegetative clone; attempts to induce flowering have failed to date. All other rice species produce seeds in Los Baños and can be distributed on request to rice scientists worldwide.

More than 100 countries have donated rice germplasm to the International Rice Genebank (Fig. 1). For many accessions without accurate passport data, we can only identify the source country from which they were received. Since 1994, with funding from the Swiss Agency for Development and Cooperation (SDC), IRRI has coordinated a major effort to collect and conserve rice germplasm from under-explored areas, in almost 30 countries, in South and Southeast Asia, Sub-Saharan Africa, and Central America. For example, there were few samples until recently from the Lao PDR in the IRGC. A geneticist from IRRI's Genetic Resources Center is based there, and has helped collect more than 10,000 samples of *O. sativa* (Appa Rao et al., 1997). Many of these samples are glutinous, upland rice varieties not conserved in any *ex situ* genebank collection, and representing a unique source of genetic diversity. Bilateral collecting activities have also been carried out in northern Australia for three wild *Oryza* species found there.

### **The Genebank**

Jackson (1997) has provided a comprehensive description of the aims and facilities of the International Rice Genebank. It has both Active and Base Collection storage vaults. For long-term conservation, seeds are dried to about 6 to 7% moisture content, and stored in vacuum-sealed aluminum cans (120g per accession) at -20°C. Laminated aluminum foil packs are used to store about 500g of seeds, and 10-g samples ready for exchange at +2°C in the Active Collection. A special seed drying room was constructed in 1994. It operates at 15°C and 15% relative humidity; seeds reach their equilibrium moisture content in about three weeks. The room has a capacity for 9,000 samples of 1 kg each. All samples are tested for germination on receipt and prior to storage after multiplication or regeneration. A regime for viability monitoring is based on initial viability estimates; japonica varieties are monitored more frequently than indica rices. The Institute's Seed Health Unit, under the supervision of the Philippines' Bureau of Plant Industry Quarantine Service checks all samples for seed-borne pathogens. Black box duplicate storage has been provided for many years by the USDA-ARS at the National Seed Storage Laboratory in Fort Collins, Colorado.

The genebank also has extensive greenhouse facilities for the multiplication of the wild rice collection. Research laboratories have been established for cytogenetics, tissue culture, and molecular studies of germplasm diversity.

### **The Policy Environment for Germplasm Conservation and Exchange**

Today, the management of the IRGC is subject to several important international policy considerations. In 1983, the FAO Commission on Plant Genetic Resources (now the Commission on Genetic Resources for Food and Agriculture) adopted the International Undertaking on Plant Genetic Resources. In the Undertaking, genetic resources were viewed as the 'common heritage of mankind.' Under the Convention on Biological Diversity (CBD) that came into force in December 1993, nations have sovereign rights over their genetic resources. The IRGC comprises both pre- and post-CBD germplasm, which are subject to very different regulations concerning access and ownership. Most accessions were acquired before the CBD, and have been designated to FAO in an International Network of *Ex Situ* Collections. Under the agreement signed between IRRI and FAO in October 1994, the trustee role of IRRI was formally recognized. However, the Institute may not claim ownership of or intellectual property rights on the germplasm accessions designated to FAO. Furthermore, these obligations are passed on to recipients of germplasm from the IRGC, through Material Transfer Agreements. Another of IRRI's obligations is conservation to the international standards adopted by FAO (FAO/IPGRI, 1994; SGRP, 1996; 1997).

### **Germplasm Multiplication and Regeneration**

Samples sent to the International Rice Genebank for long-term conservation must be multiplied to produce sufficient seeds for both the Active and Base Collections. Accessions are also regenerated if seed viability falls below 85%, or seed stocks in the Active Collection are 100g or less. Every effort is made to produce seeds of the highest viability with the minimum number of multiplication or regeneration cycles. Since all accessions are grown in Los Baños, the management of the collection in this respect presents considerable challenges for the genebank curator.

In controlled environments that simulated conditions in Los Baños (humid, tropical) and Tsukuba, Japan (temperate), we identified several factors that affect seed quality (Ellis et al., 1993; Ellis and Jackson, 1995). Then, following a series of field experiments in Los Baños, and using a broad range of *O. sativa* (both indica and japonica) and *O. glaberrima* varieties, we established protocols for germplasm multiplication and regeneration (Kameswara Rao and Jackson 1996a, b, c, 1997). The seed production cycle covers the period from late October to the end of March, coinciding with the Los Baños dry

season. Low humidity and very few rainy days characterize the dry season, and there are no typhoons that can seriously damage standing crops. Pest and disease pressures are low compared with the wet season. Rice varieties flower toward the end of December and early January, and grain filling occurs when the days are characterized by high solar radiation, and the nights are cool. Photoperiod-sensitive varieties receive a short-day stimulus at the end of December.

Accessions are harvested individually at 21 to 28 days after anthesis. Panicles are threshed in the field, and within an hour or so, seeds can be placed in the drying room. The changes to field operations, combined with the controlled drying regime ensure the production of high-quality seeds with high initial viability. This even extends to the japonica rices that are notoriously difficult to grow successfully under tropical conditions and that are less well adapted to the Los Baños environment. They have an inherently shorter storage life than indica varieties.

### Data Management

Significant improvements in the data management capability of the International Rice Genebank have resulted from advances in computer software and hardware. For many years, Oracle was used as the software platform. In 1992, development of a comprehensive data management system was begun—the International Rice Genebank Collection Information System (IRGCIS)—to include all aspects of the data flow and operations in the genebank. Initially, this was only a text-based system on the Institute's VAX 4000 to facilitate access to passport, characterization, evaluation, and seed inventory data on the more than 80,000 accessions held in the genebank at that time.

Unfortunately, the VAX 4000 was unable to support the IRGCIS with all data options installed. However, in 1997, the genebank acquired three dedicated network servers for the IRGCIS through the CGIAR's Systemwide Information Network for Genetic Resources (SINGER <http://www.cgiar.org/singer>), which holds data on the germplasm collections held in trust by 12 of the 16 CGIAR centers. We also converted IRGCIS to a Windows platform to facilitate replication to SINGER. The next phase in data management is to provide access to the system directly on the Web, in addition to access through SINGER.

Genebank staff have also developed data management systems in Microsoft Access 7.0 for several rice genebank collections in Asia, following the same principles as IRGCIS.

### Germplasm Characterization and Evaluation

Comprehensive characterization data on morphological and agronomic traits, broadly based on the IRRI-IBPGR descriptor list for *O. sativa* (IRRI, 1980), are available for almost 90% of the *O. sativa* accessions. Characterization data are collected only during the wet season, from July to October. Descriptor standardization across species was achieved to facilitate data management (especially for descriptor colors), not only for *O. sativa*. Furthermore, we now use coded states rather than actual values for many quantitative traits in *O. sativa* and *O. glaberrima*, such as culm length and number of tillers, greatly facilitating data collection in the field. The wild species are grown entirely in pots in the greenhouse, and since accurate measurements are often needed for taxonomic purposes, we continue to measure many of these traits.

Resistance to or tolerance for many pests and diseases and some abiotic stresses, such as drought and low temperatures, has been determined for many accessions in the collection, but are rather patchy in their coverage (Table 2). For some diseases like rice blast, resistance genes appear to be quite common. For others such as grassy stunt virus, resistance was found only in one accession of *O. nivara* (IRGC 101508) from India. Introduction of this resistance into *O. sativa* lines led to the release of IR36, at one time cultivated over more than 11 million hectares—the most widely cultivated food crop variety ever (Swaminathan, 1982).

A preliminary analysis of trait distribution by countries in the collection reveals several “hot spots” for resistance genes (Table 3). Careful evaluation of newly acquired germplasm from remote areas may unearth new gene combinations for pest and disease resistance. From our initial observations of morphological diversity, we are confident to find some interesting genes for pest and disease resistance in the rice varieties from the Lao PDR.

The next phase of germplasm conservation and evaluation using molecular markers such as RFLP, RAPD, AFLP, SSR, and others has begun. IRRI's collaboration with the University of Birmingham and the John Innes Centre in the United Kingdom has demonstrated the use of these markers in analyzing diversity in rice, detecting duplicate accessions, and predicting quantitative traits (Virk et al., 1995a,b; 1996). Choice of markers is critical for analysis of diversity patterns (Parsons et al., 1997), but AFLP markers are considered more robust than RAPD (Zhu et al., 1998). Anonymous markers such as RAPD and AFLP have considerable advantages over mapped markers (P.S. Virk, personal communication). These markers also differentiate indica and japonica rices according to the isozyme group classification (Glaszmann, 1987), and both have taxonomic value for the wild species. They were used to complement morphological and crossability studies of the South American AA genome species, *O. glumaepatula* (Martin et al., 1997; Juliano et al., 1998; Naredo et al., 1998; Lu et al., 1998). Tanksley and McCouch (1997) have outlined a strategy to exploit rice germplasm using molecular approaches. The next exciting phase will encompass the power of genome homoeology or synteny, at least across the grass genomes, to



identify genes of interest in germplasm collections, and thereby increase their exploitation (Devos and Gale, 1997). The development of a core collection for rice will facilitate this approach (Jackson et al., 1998).

## CONCLUSIONS

Rice is the single most important crop grown worldwide, in that it provides the daily sustenance to more people—2 billion, almost half the world's population—than any other crop. The management of the IRGC, preserving the genetic heritage of generations of rice farmers, is a responsibility that IRRI takes seriously. In accordance with international protocols, IRRI continues to make most of the germplasm from the genebank freely available, and free of charge, to researchers worldwide. New regimes for access to and use of germplasm, in accordance with the CBD, will regulate how germplasm is used in the future. Decisions taken by the FAO Commission on Genetic Resources for Food and Agriculture and the Conference of the Parties to the CBD will resolve the final status of the CGIAR center and national genebank collections that are not currently covered by the CBD. The FAO Commission recognizes the importance of the CGIAR germplasm collections. As part of the FAO International Network of *Ex Situ* Collections, IRRI will ensure that the world's largest collection of rice genetic resources continues to contribute toward the dual goals of food security and alleviation of poverty.

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**Table 1. Composition of the International Rice Genebank Collection at the International Rice Research Institute, 14 August 1998.**

Species	Genome	No. accessions
<i>O. sativa</i>	AA	79,925
<i>O. glaberrima</i>	AA	1,244
Non-registered cultivated samples	AA	16,869
<i>O. barthii</i>	AA	225
<i>O. glumaepatula</i>	AA	38
<i>O. longistaminata</i>	AA	155
<i>O. meridionalis</i>	AA	43
<i>O. nivara</i>	AA	549
<i>O. rufipogon</i>	AA	800
<i>O. punctata</i>	BB, BBCC	55
<i>O. minuta</i>	BBCC	67
<i>O. officinalis</i>	CC	266
<i>O. rhizomatis</i>	CC	19
<i>O. alta</i>	CCDD	10
<i>O. grandiglumis</i>	CCDD	10
<i>O. latifolia</i>	CCDD	37
<i>O. australiensis</i>	EE	25
<i>O. eichingeri</i>	EE	25
<i>O. brachyantha</i>	FF	18
<i>O. granulata</i>	GG	23
<i>O. meyeriana</i>	GG	8
<i>O. longiglumis</i>	HHJJ	6
<i>O. ridleyi</i>	HHJJ	15
<i>O. schlechteri</i>	-	1
Hybrids/unidentified	-	670
Oryzae species	-	15
Non-registered wild species samples	-	1,665
<b>Total accessions and samples</b>		<b>102,783</b>

**Table 2. Resistance to biotic and abiotic stresses among accessions in the International Rice Genebank Collection.**

Stress	No. accessions tested	Accessions resistant (%)
Rice blast	36,293	42.0
Bacterial blight	48,079	7.6
Sheath blight	22,770	9.3
Brown planthopper–biotype 1	44,504	2.2
Brown planthopper–biotype 2	10,808	4.2
Brown planthopper–biotype 3	492	3.8
Whitebacked planthopper	52,513	2.6
Yellow stem borer	16,127	4.4
Striped stem borer	7,214	<1.0
Green leafhopper	50,314	3.7
Zigzag leafhopper	3,125	17.8
Rice leaf folder	8,825	<1.0
Rice whorl maggot	23,120	3.1
Drought tolerance 4*	28,095	15.2
Drought tolerance 5	22,637	8.1
Drought tolerance 6	11,515	4.3
Alkali tolerance	2,653	38.8
Cold tolerance	6,638	9.4
Salt tolerance	8,009	11.6
Zinc deficiency 1**	962	4.8
Zinc deficiency 2	119	10.9

\* Drought tolerance 4, 5, and 6 are different values scored at the active tillering, late vegetative, and reproductive stage, depending on the maturity of the variety.

\*\* Zinc deficiency 1 and 2 are scored in a zinc deficient field or a screenhouse test, respectively, at 4 and 6 weeks after transplanting

**Table 3. Country “hot spots” for resistance genes among germplasm accessions in the International Rice Genebank Collection.**

Disease	Country
Bacterial blight	Bangladesh, Philippines
Blast	Lao PDR, Myanmar, Thailand, Vietnam
Sheath blight	Malaysia, Sri Lanka, Vietnam
Rice tungro	Bangladesh

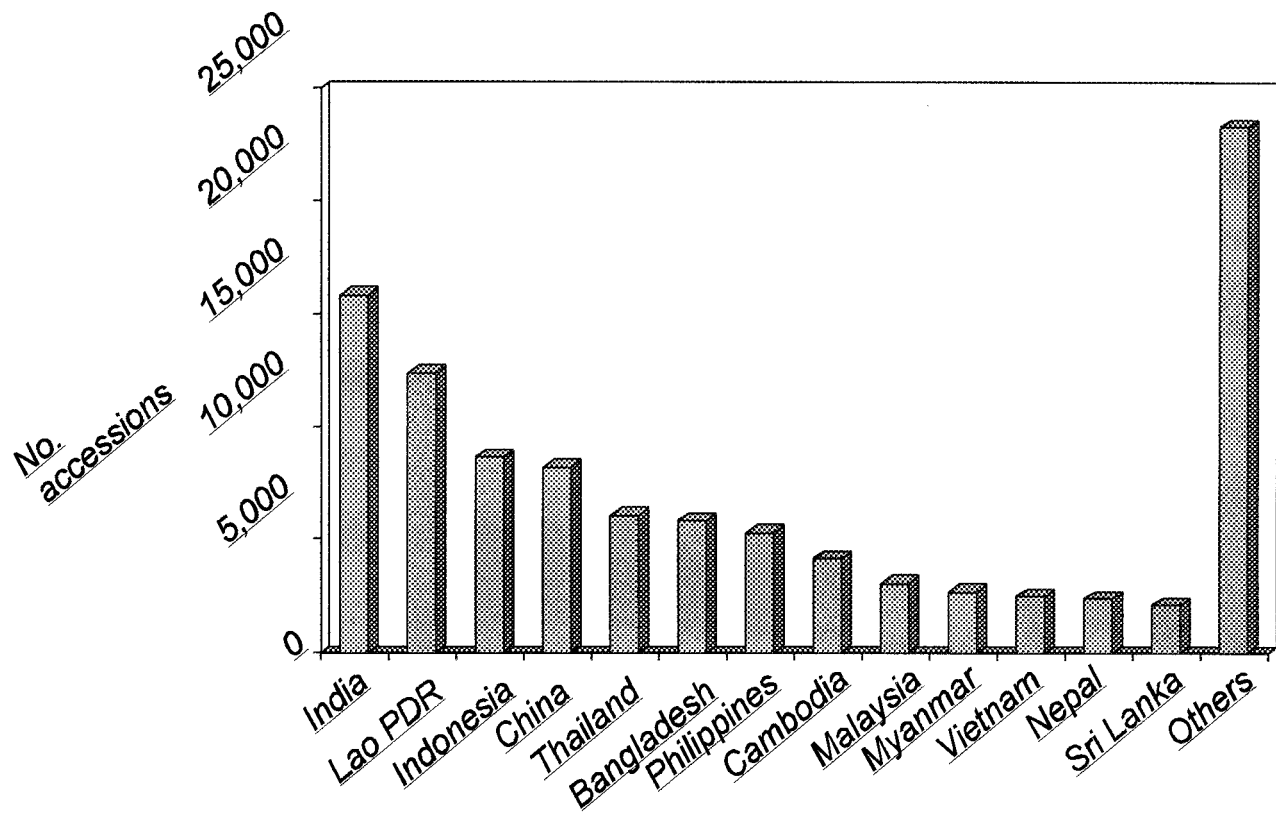


Fig. 1. The source of germplasm in the International Rice Genebank at IRRI.

## RICE GERmplasm EVALUATION AND ENHANCEMENT AT WARDA

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### ABSTRACT

For more than three decades, breeding research programs have targeted constraints limiting yields to improve the performance of rice varieties. While the products of this research outperform farmer's traditional varieties under relatively high input systems; their performance under low inputs that dominate rice farming in West Africa has been poor.

Rice scientists at WARDA systematically evaluate germplasm from both within and outside Africa. The strategy has been to combine specific adapted attributes of local rice varieties with the yield potential of introduced varieties. During the period of 1994-97, the Germplasm Exchange unit at WARDA distributed to NARS breeders in West Africa, 207 nurseries for a total number of 17,872 varietal requests.

WARDA launched an innovative interspecific crossing program between Asian rice, *O. sativa* and African rice, *O. glaberrima* in 1991 to develop weed competitive and highly input responsive, but not input-dependent, rice types for various water limited ecosystems. Research characterizing the traits of the indigenous *O. glaberrima* and its wild relatives has shown a reservoir of useful genes for resistance to various stresses. With the identification of suitable parental varieties, WARDA has covered another milestone in widening the genetic base of the West African rice germplasm by successfully introgressing useful genes from indigenous *O. glaberrima* into *O. sativa*.

Evaluation of the new progenies showed considerable promise as progenies with high yield potential, high levels of resistance/tolerance to stresses such as weeds, drought, acidity and blast have been identified for further evaluation by NARS, development agents and farmers in participatory research. The interspecific progenies might provide solutions at two levels, the improvement of labor productivity through reduced need for weeding and other management interventions; and the improved sustainability of intensified systems through durable crop resistance to pests and diseases, particularly in lowland systems that so far depends on introduced materials.

Working in close partnership with national scientists, development agents, farmers and policy makers, significant impact is expected on farmer's fields before the end of this decade with increasing momentum in the next century.

### INTRODUCTION

Rice is the main staple food in at least 8 of the 17 countries of West Africa and is rapidly gaining popularity as a major food in the others. However, self-sufficiency in West African rice production is declining because demand is increasing faster (5.9% annual growth since 1970) than its production (4% annual growth over the period).

Rice growth environments in West Africa are extremely diverse, varying as a function of agro-ecological, geo-morphological, hydrological, and socio-economic factors.

Rainfed rice is the predominant production system in West Africa, occupying approximately 80% of rice area and producing 75% of regional production (Table 1). Though exact statistics are not available, it is estimated that over 80% of the resource poor rice farmers in West Africa grow rice as a subsistence crop. Most rice farmers in West Africa grow land races which are generally tolerant to environmental stresses but whose yield potential is lower than that of improved varieties. On the other hand, improved varieties bred for improved management conditions generally are not well adapted to traditional farming systems. New improved rice varieties better adapted to the resource poor farmer's conditions are needed that draw on existing germplasm or are developed by combining desired characters from various germplasm sources.

Crop improvement scientists at the West Africa Rice Development Association (WARDA) systematically evaluate germplasm from both within and outside Africa, generate breeding materials, select superior lines and test early and advanced breeding materials on-station and onfarm. WARDA's strategy for rice improvement is to combine specific agro-ecological adaptations of local rice varieties with the yield potential of introductions.

Targeting the numerous constraints limiting yields of rice such as drought, weeds, blast and low input cultural practices, conventional breeding programs have been active over more than three decades to improve the performance of rice varieties in West Africa. The gains from this research have been limited, in part because *Oryza sativa* L., the most widely cultivated rice species in West Africa, has limited resistance to many of the stresses that affect rice in the region. Although advanced selections from these intra-specific breeding programs mostly out perform farmers' traditional varieties on-station under relatively high input conditions, they perform poorly when cultivated under the low input systems which dominant rainfed rice farming in West Africa. In an effort to break this pattern, WARDA initiated interspecific hybridization program in 1991 to introgress important traits between *O. sativa* and *O. glaberrima* thereby, increasing the genetic variability within each type.

Rice scientists have been aware of *Oryza glaberrima* for many decades. Among eight other species indigenous to Africa (Table 2), *O. glaberrima* is known to have been selected and cultivated in parts of West Africa more than 3500 years ago. Because *O. glaberrima* has survived without help, or interference of man, the species has developed adaptive or protective mechanisms for resisting major biotic and abiotic stresses. *O. glaberrima* represents a rich reservoir of useful genes for resistance to diseases and insect pests as well as tolerance to acid soils, iron toxicity, drought, unfavorable temperatures and excess water (Table 3). This paper present approaches for germplasm development and dissemination at WARDA.

## PROCEDURES

### Constraints to Rice Production

The socio-economic, biological, and environmental constraints to increased rice production in West Africa can be summarized as follows:

**Socio-Economic Constraints:** The socio-economic constraints to increased rice production are common across West Africa environments, and affect the agricultural sector as a whole rather than just rice. Despite the reforms being introduced by structural adjustment programs, there remain marked deficiencies in pricing and marketing policies in many countries of the region. A general lack of infrastructure including roads, vehicles, input supplies centers, and grain storage facilities constrain both the availability of inputs and marketing of output. Weeding and harvesting are generally affected by shortage of labor and inappropriate machinery. This also hampers threshing, drying, storage, and milling of rice. Most upland rice farmers are poor and accordingly use little or no fertilizer, no insecticide, and no fungicides.

**Biological and Environmental Constraints:** Rice is grown in such diverse conditions that a complete list of biophysical constraints, which could be targeted by plant breeding, is difficult. A broad array of biotic and abiotic constraints that combine to reduce rice yields in West Africa were identified by CARS and WARDA scientists in 1991. Priority (severity) ratings of high, medium, and low corresponding to the degree of severity of the constraints were endorsed and used to score each constraint by CARS representatives (17 countries in West Africa). Data for each country, and eventually for the region, were then obtained. The constraints that cut across countries in West Africa include: periodic drought; weeds; diseases such as blast, rice yellow mottle virus, glume discoloration, leaf scald, brown spot, sheath rot, and sheath blight; insect pests such as stem borers, the African rice gall midge, defoliators, grain sucking bugs, and nematodes; and acidic soils deficient in nitrogen (N) and phosphorus (P), and with toxic levels of iron (Fe), aluminum (Al), and manganese (Mn). We should note that weeds pose a double constraint. The ability to remove weeds before seeding largely determines the area that can be grown by family labors and the efficiency of weeding after sowing greatly affects grain yield harvest.

### Rice Relatives in the Afro-Tropical Regions

Twenty species of rice have been identified with the genus *Oryza*, and of these, eight are indigenous to Africa (Table 2). In addition to the indigenous domesticated species, *O. glaberrima* grown in West Africa, the Asian domesticated species, *O. sativa* is widely grown in Africa. The wild species of *Oryza* indigenous to Africa are *O. barthii*, *O. longistaminata*, *O. punctata*, which is both diploid and tetraploid (Chang and De Datta, 1975; Ng et al., 1983). The chromosome number, genome grouping and geographical distribution are as indicated in Table 2.

### Rice Improvement Activities at WARDA

The goal of WARDA's varietal improvement research is to develop high and stable yielding rice varieties well adapted to target ecosystems. We believe that majority of the rainfed rice farmers are likely to continue with traditional low-input cultivation systems in the short to medium term. For this reason our goal is to develop varieties that perform better than farmers' traditional germplasm under low management levels, but that are also more responsive to additional nitrogen, water, and labor so as to encourage greater input use. The new varieties should also have tolerance equal to or superior to

most traditional varieties for drought, adverse soils, insect pests, and diseases. Finally, they should show appropriate architecture/plant type to resist lodging and suppress or tolerate weeds, and grain quality properties matching consumer preferences.

Activities of WARDA's varietal improvement research are detailed in its medium-term plan (1998-2000) that was approved by TAC in May 1997. Research is implemented by interdisciplinary teams of scientists in breeding, entomology, pathology, agronomy, soil science, economics, and grain quality analysis, with the close collaboration of CARS breeders under the auspices of the Rice Breeding Task Forces.

Principal Breeding Objectives: Although some general breeding goals are common to most rice breeders in West Africa, each country has more specific breeding objectives especially in relation to particular adverse soil, disease and insect pest problems. The principal breeding objectives at WARDA are:

- **High Yield:** Upgrade yield potential by developing stiff, non-lodging, intermediate statured, and moderately tillering plant types to replace tall, lodging susceptible traditional varieties. Plants should be moderately tall with moderately long, droopy lower leaves, particularly at the vegetative phase, to compete with weeds.
- **Yield Stability:** Enhance resistance to blast, the rice yellow mottle virus, the African gall midge, sheath rot, brown spot, leaf scald, stem borers, and diopsis. Enhance resistance or tolerance to drought and drought recovery ability. The latter of which is generally poor in traditional varieties.
- **Growth Cycle:** Develop a range of maturities to suit various rainfall and cropping patterns. Weak photoperiod sensitivity is required in some areas with high initial rainfall so that the maturity phase coincides with periods of relatively low rainfall.
- **Adverse Soils:** Retain or incorporate genes for high N and P uptake and use efficiencies, and tolerance to salinity, Fe, Al and Mn toxicities in acid soils.
- **Panicle, Grain Type, and Quality:** Retain preferred agronomic characteristics: long, well exerted panicle, high panicle fertility, nonshattering spikelets, and grain quality (good aroma and taste, intermediate to high amylose, medium gelatinization temperature, high protein content, and favorable amino acid balance). Specific grain size and shape are secondary importance, as they are less rigidly preferred by West African consumers.

Partnership with CARS in Rice Improvement: Individual CARS in West Africa vary considerably in strength. Some are capable of conducting basic and applied technology generating research, but the majority is capable of mounting only adaptive and testing programs. To have significant impact WARDA requires the collaboration of strong national programs both in technology generation and to adapt results to particular rice growing environments. Moreover, a greatly strengthened capacity and cooperation among national programs must ultimately meet the long-term needs for rice research in the region.

For this reason, WARDA posits partnership with its Member States as one of its basic institutional values, and the strengthening of their research capabilities as one of its principal objectives. We have operationalized the concept of partnership through a Task Force approach by which WARDA strengthens CARS while catalyzing collaboration among the region's diverse rice research institutions.

Task Forces are mini-networks that bring together regional scientists who are working on very similar research problems in similar rice growing environments. During 1990 to 1991, four varietal improvement Task Forces were created. The aim of each of the four Task Forces, is to execute a fully complementary work program by assembling scientists engaged in rice breeding to transfer skills, information, and germplasm among CARS and between CARS and WARDA. This involves close consultation with CARS to reach consensus on the priorities to guide collaborative regional rice research, to review WARDA and CARS research activities and results, and plan the strengthening of CARS research capacity.

### **Genetic Resources Activities**

Germplasm Collection: Several organizations in West Africa, i.e. Office de la Recherche Scientifique et Technique d'Outre-Mer (ORSTOM), Institut de Recherches Agronomiques Tropicales et des Cultures Vivrières (IRAT), Institut des Savanes (IDESSA), International Plant Genetic Resources Institute (IPGRI), International Institute of Tropical Agriculture (IITA), and WARDA have been actively collecting rice germplasm (*Oryza* species) in West Africa, during the past several years.

The WARDA germplasm program started in 1978 following a collaborating agreement signed by WARDA, IRRI, IITA, and IRAT for germplasm collection in West Africa. The initial activities of the program centered on:

- Compilation of information from member states on the status of rice germplasm collection in the region.
- Maintenance of a working collection at WARDA's main research stations.
- Receiving seed samples of collections from IITA, IRAT, ORSTOM, and other organization.

Some major accomplishments in the WARDA germplasm collection in West Africa are as follows:

- In 1979, WARDA, with the assistance of the Indian Council of Agricultural Research, carried out expedition in Mali, resulting in the collection of 666 accessions belonging to five species: *O. glaberrima* (314), *O. sativa* (332), *O. longistaminata* (10), *O. barthii* (7), and *O. stapfii* (3).
- In 1985, WARDA and a team of explorers from the Faculty of Agriculture of Kagoshima University, Japan, collected 23 indigenous cultivars of *O. sativa* and *O. glaberrima* in Liberia.
- On a continuing basis, WARDA and CARS scientists collect new accessions during field visits in 17 WARDA member countries in West and Central Africa. CARS scientists also forward seed samples of new collections to WARDA. The following are some examples:
  - Between 1978 and 1980, WARDA scientists at the Mangrove Swamp rice program in Rokupr, Sierra Leone, collected 507 traditional mangrove swamp rice varieties from the Gambia, Guinea, Guinea Bissau, and Sierra Leone (WARDA 1980). By 1986, the collection stood at 754 accessions of mainly *O. sativa* and few *O. glaberrima* from the Gambia, Guinea, Guinea Bissau, Nigeria, Senegal, and Sierra Leone. Duplicate samples of the accessions were sent to IRRI, Los Banos, Philippines.
  - The collection made in Mali during late 1979, covered deep-flooded and floating rice areas in the country: Mopti (Kouna, Koloni, Bussoura, Segue, Ngomo, Sareseni, Tenkry, and Diambacourou), Gao (Barons, Garoua, and Goutchien) and Tenekou (Daga, Boukani, Diondioni, Diafarabe, Kara, and Koubi). A total of 104 varieties were collected. The collection was made up of *O. sativa*, *O. glaberrima*, *O. barthii*, and *O. stapfii* species.
  - The WARDA Rained Program at Bouakè in Côte d'Ivoire also collected and received upland rice varieties from CARS between 1985 and 1997. A good number of accessions were received from IRA, IOTA, and ORSTOM. Over 16,000 accessions of *O. sativa* (14,500), *O. glaberrima* (1,500), *O. longistaminata* (121), *O. barthii* (800) and *O. stapfii* (3) species are retained as working collection at the WARDA Bouaké cold room. Five thousand accessions have been designated and place under the auspices of the FAO.

Most of the major rice growing countries in West Africa have been explored during the past several years. The countries explored include Benin, Burkina Faso, Cameroon, Chad, Cote d'Ivoire, The Gambia, Guinea, Guinea Bissau, Liberia, Mali, Niger, Nigeria, Senegal, Sierra Leone, and Togo. Many remote areas in each of these countries, and others like Mauritania, need to be further explored. Further collection of rice germplasm in West Africa is, however, hindered by financial constraints and assistance is urgently needed to organize formal expeditions to remote areas in the region. It should be noted that valuable germplasm are being eroded annually due to overgrazing, uncontrolled land clearing and perennial bush burning which characterize African agriculture. This makes it difficult to practice *in situ* conservation in the region. Also on-farm conservation of species is not well developed in West Africa because of the difficulty of monitoring on-farm operations and the inadequate seed storage facilities at the farmers' level. Farmers however conserve as much seed as is needed for planting during the following season.

Germplasm Conservation: WARDA's accessions are currently stored as working collections at each of two research stations. The program at Bouaké, Côte d'Ivoire, is conserving over 14,000 seed samples of upland, rained lowland, deep water and irrigated rice varieties. The program at St. Louis in Senegal is maintaining about 2,000 irrigated rice varieties. For conservation, seed is produced at each of WARDA's main research stations during the post-rainy season when good quality disease free seed can be produced. Experts inspect the standing crop and only disease and insect free seed is harvested. Moisture content is brought down to 6 to 8% before storage in the cold room. At each station 500 to 2,000g seed sample of each accession is kept in appropriate containers in air-conditioned rooms with temperatures ranging from 18 to 20°C and relative humidity from 20 to 30%.



### Germplasm Evaluation and Use

**Diversity of African Rice Germplasm:** Four of the six genomes known in the genus *Oryza* are native of Africa (Ng et al., 1993). The majority of the *Oryza* species particularly, those with AA genome (Table 2) are crossable with cultivated rice, *O. sativa*, although seed sets are very low. Through isoenzymatic electrophoresis carried out by ORSTOM of France it has been shown that new polymorphisms exist within *O. sativa* traditional land races and introgression with other species like *O. longistaminata* is common among the land races of rice (Charrier and Hamon, 1991). This indicates that natural introgression of the relative species into *O. sativa* has taken place in West Africa over several years.

Success in plant breeding depends on the diversity of the genetic resources for genes responsible for various traits. Because the wild species have survived without help, or interference of man, they have evolved mechanisms for resisting pests, diseases, and other environmental stresses. The wild germplasm, therefore, is a reservoir of useful genes for resistance to diseases, and insect pests, and tolerance for biotic stresses (iron toxicity, drought, unfavorable temperature conditions, excess water etc.); increased biomass and grain yield; and improved grain quality.

**Morphological Characterization of Germplasm:** Characterization and evaluation of available germplasm have been partly done at WARDA based on phenotypic characteristics. The characterization work showed that there is very wide variation in important morphological and agronomic traits within the traditional land races of *O. sativa* and *O. glaberrima* (Table 3), and some wild rice populations. These include rapid and vigorous vegetative growth to suppress weeds, tolerance/resistance to major stresses such as drought, blast, African rice gall midge (ARGM), rice yellow mottle virus (RYMV), nematodes, and soil acidity. For example:

- Considerable variation in seedling vigor was observed within each varietal group evaluated at WARDA between 1992 and 1997. However, over 90% of the 1,300 *O. glaberrima* screened had very high seedling vigor, and 15% also had very rapid vegetative growth and produced a large number of tillers and droopy lower leaves within 20 to 30 days after sowing, resulting in rapid ground cover and good weed suppression. It was also noted that *O. glaberrima* had the ability to compensate for weed competition in the early stages of crop growth by late tillering 30 to 60 days after the crop had been weeded.
- Among the improved *O. sativa* screened, the distribution of growth duration (days from planting to maturity) tended to cover a narrow range with most entries between 80 to 120 days. In the *O. glaberrima* accessions there was a much wider range (about 85 days between the minimum and maximum values) and several with very early duration of between 65 to 75 days to maturity were identified confirming report by IRRI that some accessions of *O. glaberrima* evaluated during the dry season at Los Baños, Philippines matured within 50 to 60 days. The merit of early-maturing rice varieties is that they may escape drought in drier areas, while in more humid areas they will escape late-season fungal diseases such as blast, glume discoloration, and sheath rot.

**Breeding for Specific Traits:** Some progress has been made in breeding for specific traits such as disease and insect pest resistance, tolerance for nutritional deficiencies, soil acidity, and salinity, Fe, Al, and Mn toxicities, and drought resistance.

- Drought resistance: Over 2,500 *O. sativa* (1,900) and *O. glaberrima* (600) germplasm evaluated at WARDA between 1991 and 1994 for resistance to drought showed considerable variation for seedling vigor, tillering ability, leaf tip burn and leaf rolling, and unrolling ability. Several land races of *O. glaberrima* and a few *O. sativa* with resistance at the vegetative and/or reproductive stages of growth were identified and used in the breeding program. Interspecific progenies from *O. glaberrima* and *O. sativa* crosses are showing rapid vegetative growth and good seedling vigor during drought stress at the vegetative stage. They are also showing high recovery ability after relief of the stress.
- Blast resistance: Of 123 *O. glaberrima* entries screened in 1993 at WARDA for resistance to leaf and neck blast which occurs in most rice-growing areas in West Africa, 14 were rated as resistant. Several resistant entries, including TOG6701, TOG7264, TOG6690, and TOG7386, had symptoms similar to those in *O. sativa* landraces, OS6, Moroberekan, Lac 23 and 63-68.
- RYMV resistance: Forty-four accessions of *O. glaberrima* inoculated with the RYMV were found to be highly resistant. Also at IRRI, Los Baños, Philippines, out of 272 strains of *O. glaberrima* tested for resistance to rice tungro virus, 44 were resistant.

- ARGM resistance: Of 586 *O. glaberrima* accessions screened between 1994 and 1996 for resistance to the African rice gall midge and Hospet which can devastate rice plants during the vegetative stage in hydromorphic and lowland conditions, 13 had a high level of resistance and in a separate stemborer test, 61% of the 586 *O. glaberrima* was rated as resistant.
- Acidity tolerance: Several *O. glaberrima* varieties with moderate levels of tolerance to acidity, low P and iron toxicity have been identified and are being used in the breeding program.
- Salinity tolerance: Several improved varieties introduced from Asia have been screened for salinity tolerance and adaption to Sahel irrigated conditions. Promising lines have been identified and are being used in hybridization with traditional salt tolerant varieties.
- Resistance to striga: Three months after sowing 40 lines of *O. glaberrima* and *O. sativa* check varieties in the experiment to screen for hosts of the parasite weed *Striga aspera* and *S. hermonthica* at WARDA, the *O. sativa* lines were highly infested with both. However, 18 *O. glaberrima* lines remained free of *S. aspera* and three appeared to be resistant to *S. hermonthica*.

The above findings showed that *O. glaberrima* and the wild species are valuable sources of an array of useful but unexploited genes for resistance to major stresses, while most cultivars exhibit susceptibility to the prevalent diseases and pests. Therefore, wide crosses of rice involving intragenomic and intergenomic hybridization with relative species native to Africa have great potential for widening the genetic base for the subsequent development of improved varieties suited to the prevailing biotic and biotic as well as socio-cultural conditions in West Africa.

### Interspecific Hybridization

Aware of the rich potential that lies within the *O. glaberrima* genes, but also conscious of the difficulties that have frustrated past efforts to unlock this potential treasure, WARDA breeders launched a major new wide crossing program in 1991. *O. glaberrima* parents that had the best combination of characteristics were selected. In 1992, these lines were used in a wide hybridization program with the best *O. sativa* upland rice varieties that had been developed by WARDA. Conventional backcrosses, anther culture and embryo rescue techniques were used to break the sterility barrier.

The successful introgression of genes from *O. glaberrima* into *O. sativa* has not only resulted in a significant broadening of the genetic base of cultivated rice but has also produced new plant prototypes with extremely interesting agronomic traits. The new plant type combines good resource and input responsiveness with high yield stability under a broad range of upland and hydromorphic conditions. Specifically, it has the following properties.

- In the vegetative growth phase, it possesses weed competitiveness through its superior growth vigor, rapid N uptake, drought resistance, and rapid ground cover (through its droopy leaves, high partitioning of C to leaves, and high tillering ability).
- In the reproductive/ripening growth phase, it has high assimilate sink capacity (through its secondary panicle branches and high panicle number), high assimilate source capacity (through its thick dark green leaves, erect leaves, and stiff culms), and high harvest index (through its intermediate height, big panicles, lodging resistance, and resistance to shattering).
- It shows resistance both to biotic (weeds, blast, nematodes) and biotic stresses (soil acidity, P deficiency, drought).

### Diffusion of Elite Breeding Lines to National Scientists and Farmers in West Africa

The dissemination of the new lines to national programs in the region is done through two applied and adaptive research mechanisms: (1) regional testing of fixed lines and (2) farmer participatory varietal selection studies.

WARDA collaborates with national program researchers through the four varietal improvement Task Force (i.e. upland, rainfed lowland, irrigated lowland and mangrove swamp rice Task Forces). For example, during the period of 1994-97, the Germplasm Exchange Unit of WARDA distributed to CARS breeders in West Africa, 207 nurseries for a total number of 17,872 varietal requests. In 1997 alone, over 5,000 promising cultivars including 600 interspecific progenies were distributed to collaborating scientists in Asia, Latin America and national programs in Africa as well as to inhouse scientists as part of WARDA's activity on germplasm sharing.

New approaches to technology development and dissemination are being followed in WARDA to facilitate farmer's involvement in the processes. These are the Farmer Participatory Varietal Selection (PVS) and Farmer Participatory Rice Breeding (PRB) approaches. These studies allow for accelerated diffusion of new lines including interspecific progenies as farmers themselves take the lead in selecting acceptable materials using their own criteria. The approaches also help ensure that cultural practices that accompany the new varieties are well adapted to farmer's conditions and thus subject to early and broad adoption. In 1997, PVS and PRB trials were conducted under rained conditions in four countries: Côte d'Ivoire, Ghana, Togo, and Guinea. These trials have been extended to six other countries in West Africa during 1998. Under Sahel irrigated conditions the best selections from on station trials are transferred to the production sector through participatory research. Through this exercise three varieties, Sahel108, Sahel201, and Sahel202 have been released in Senegal, Mauritania, and Burkina Faso in the last four years.

In general, the PVS exercise has proved very popular with farmers. The approach has already paid handsome dividends, besides speeding up the development of new varieties, it is helping to gauge the acceptability of materials as well as stimulating demand for new varieties.

### **Training**

One of the aims of the varietal improvement project and in line with WARDA's mandate is to ensure the development of adequate national capacity in the region to use the emerging genetic resources and also share in the benefits arising from their use. It also aims at establishing effective collaborative arrangements and networks. Being the only laboratory in the region with such facilities, CARS personnel are being trained at WARDA on anther culture methods and handling of interspecific progenies. Forty-two scientists from 16 countries received training in participatory research methods and/or rice improvement methods and genetic evaluation and use at WARDA for one to two weeks in 1997. Visiting scientists to WARDA interspecific breeding program were received from Sierra Leone (2) Cote d'Ivoire (4), Guinea (10), and Togo (1) during 1997.

### **Future Emphasis**

In the past two decades, considerable progress has been made in identifying and developing rice varieties with fairly high yields, and with genetic resistance/tolerance to major biotic and environment stresses. Although hundreds of improved rice varieties are available, most resource-poor farmers are still holding on to their traditional varieties because they perform better under unfavorable rained conditions. Efforts have been made to develop new improved rice varieties for distinct levels of management in the last few years by WARDA, in collaboration with its CARS Task Force partners.

Emphasis in WARDA's rice improvement research has shifted during the last 10 years from introduction and selection of promising varieties toward hybridization. WARDA's hybridization program has been greatly expanded to include wide crossing between *O. sativa* and *O. glaberrima* and its wild relatives and to generate more and diverse materials, especially for the resource-poor farmers in the different rice environments of West Africa. Efforts are also being made to transfer through molecular biology genes for RYMV and ARGM resistance from *O. glaberrima* to *O. sativa*.

The collaborative breeding effort between WARDA and CARS, and among the CARS, under the umbrella of the varietal improvement Task Forces, and with other institutions such as IRRI, CIAT, ORSTOM, and Cornell University will be strengthened. In all cases, there should be more intensive exchange of information and materials not only of varieties but also of early segregating populations to facilitate the selection germplasm will adapted to the requirements of specific environments, which otherwise may be lost, if only fixed lines are distributed.

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**Table 1. Rice production systems in West Africa: Extent and potential**

Ecosystem	Cultivated area		Potential yields		Target average (Tons/ha)
	Ha (mil)	%	Unfavorable ------(Tons/ha)-----	Favorable	
Upland	1.7	39	1.5	4.5	2.0
Rained Lowland	1.6	38	2.5	5.0	3.0
Irrigated	0.5	12	5.0	7.0	4.0

**Table 2. The Species of *Oryza* in Africa.**

Species	Chromosome number	Genome	Origin	Useful traits
<i>O. sativa</i> (cultivated)	24	AA	Asia	High grain yield
<i>O. glaberrima</i> (cultivated)	24	AgAg	West Africa	Early vegetative vigor, drought tolerance, resistance to green leafhopper (GLH)
<i>O. stapfii</i> (weed species)	24	AgAg	West Africa	
<i>O. barthii</i>	24	AA	West Africa	Drought avoidance, bacterial blight resistance
<i>O. longistaminata</i>	24	AA	Africa	High pollen production, long stigma, drought tolerance, source of cytoplasmic male sterility
<i>O. brachyantha</i>	24	FF	East and Central Africa	Tolerance to lateritic soils, resistance to yellow stemborer, leaf folder, and whorl maggot
<i>O. eichingeri</i>	24	CC	Tropical Africa	Shade tolerance, resistance to brown planthopper (BPH), GLH, and white backed planthopper
<i>O. punctata</i>	48	BBCC	Tropical Africa	BPH resistance

**Table 3. Range of variation of some selected characters of *O. sativa* and *O. glaberrima* recently characterized for 44 traits at WARDA Mbé station.**

Character	<i>O. glaberrima</i>	<i>O. sativa</i> (Traditional)	<i>O. sativa</i> (Improved)
Seedling vigor (days after sowing) <sup>a</sup>	1-7	1-7	1-7
Plant height (cm)	65-138	80-182	65-138
Growth duration (d)	75-160	105-150	75-160
Panicle m-2(no.)	46-470	6-476	49-477
Grain length-to-width ratio	2-3	2-3	2-3
Panicle fertility (%)	40-98	50-98	60-90
1,000-kernel weight (g)	6-38	6-38	13-39

## RICE GERMPLASM EVALUATION AND ENHANCEMENT AT CIAT

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### ABSTRACT

Over the last 30 years, 295 varieties were released in Latin America and the Caribbean, mainly originating from crosses made at CIAT. As a result, large increases in yield and production occurred to the benefit of both consumers and producers.

Rice breeding at CIAT continues to concentrate on the development of germplasm for irrigated (the main focus) and rainfed lowlands and for favored uplands of Latin America and the Caribbean. Research focuses on following traits: yield improvement; durable resistance to blast; resistance to the rice “hoja blanca” virus and its vector, grain quality, iron toxicity tolerance, adaptation to acid soils, cold tolerance, and allelopathy; and anaerobic vigor for better weed control. The breeding methods used to broaden the genetic diversity of rice include pedigree selection, backcrossing, and recurrent selection. Molecular tools, mainly anther culture, molecular markers, DNA fingerprinting, and genetic transformation, are used to characterize pathogen variability; identify, tag, and transfer genes conferring resistance/tolerance to problems of regional importance. Research on population development for yield enhancement includes testing and crossing the IRRI new plant type and wild species of rice with high-yielding commercial varieties. The CIAT Rice Project concentrates on developing gene pools in collaboration with the International Network for Genetic Evaluation of Rice for Latin America (INGER-LAC). It complements the Fund for Latin America and Caribbean Irrigated Rice (FLAR), which focuses on variety development.

### INTRODUCTION

Current world rice production is around 570 million tons of paddy, of which Latin America and the Caribbean (LAC) produce 3.6%, and plant 4.1% of the total area. The area harvested in rice in Latin America is around 6.1 million ha. In 1995, flooded rice occupied 54.5% of the area and upland rice 45.5%. Per capita consumption of white rice increased from 10 kg in the 1920s to about 30 kg in the 1990s and is now the most important cereal for human consumption across most of the tropics of LAC.

Global production of rice has outpaced population growth over the past three decades mainly due to important gains in productivity. Several indicators confirm these impressive results: higher per capita rice supplies, substantial yield increases and lower prices available to consumers. Increased productivity implies that millions of hectares were not disturbed (e.g. forests) to feed a rapidly growing population. This has been accomplished, to a large extent, through the relentless search for better varieties. This task was facilitated by the availability of new and alternative tools for research, increased scientific knowledge, particularly in the field of molecular genetics, and vastly improved communications. Although the investment in rice research over the past three decades was large, returns from this investment have been huge.

CIAT's comparative advantage in rice research lies in addressing those constraints and opportunities, which are distinctive to LAC. Rice hoja blanca virus (RHBV), for example and its insect vector are present only in LAC. Some pests like rice blast are common in LAC, Africa, and Asia, but virulence diversity and frequencies are different. Direct seeding of irrigated rice is more important than transplanting in LAC, in contrast to Asia and Africa. Higher labor costs and less well developed water control in LAC as compared to Asia, exacerbates weed and disease problems.

#### **Impact of Rice Research in Latin America and the Caribbean.**

Rice production in LAC over the 1990-97 period increased at an impressive annual rate of 3.8%, the highest of all rice-growing regions in the world. This was spurred almost entirely by productivity gains through the use of new, high-yielding varieties and by a shift to more productive ecosystems (from upland to flooded). Total rice production doubled between 1967 and 1995 to reach about 20 million tons of paddy rice, making the region self-sufficient.

Over the period 1967-1998 some 295 new rice varieties were released in LAC (or about 10 per year), the majority of them (90%) targeted to flooded environments. Of the newly released varieties, 40% originated from crosses made at the

International Center for Tropical Agriculture (CIAT), 11% from IRRI and many of the remainder have parents developed by either IARC (Table 1). Modern semi-dwarf varieties (MSVs) now account for 93% of all flooded rice, representing more than 80% of total rice production in the region (Table 2). Average yield in flooded areas have risen from 3.3 tons per hectare in the mid-1960s to 4.6 t/ha in 1995.

During LAC's rapid urbanization, 70% of the population now lives in cities, rice has displaced the diets containing traditional, bulky, and perishable staples. With rice prices falling by about 50% in real terms over the last 30 years as a result of research, consumers have been the main beneficiaries. Rice has become the most important source of calories and proteins for 20% of the population with the lowest incomes. Consumption increased over the last 15 years even in high level consuming countries such as Ecuador, the Dominican Republic, Cuba, Peru, Brazil, and Colombia.

Parallel to these accomplishments were important institutional developments:

- Progressive involvement of the private sector in rice research and extension through formal strategic alliances with public institutions, both national and international.
- Through CIAT a strong linkage of the region was established with IRRI, the world's premiere source of rice germplasm
- Close cooperation between CIAT's regional rice program, national programs and producers in the major rice producing countries of LAC

### **Present and Future Research Strategies**

The past three decades have resulted in strong national rice improvement programs, high-yielding rice varieties in farmer's fields, and operational networks of germplasm improvement linked with CIAT and IRRI. In the mid-1990s CIAT's Rice Program evolved from the breeding and agronomy approach into the Rice Project with a focus on prebreeding activities that complement the varietal development and training activities of the new, private funded partnership that emerged in 1995: the Fund for Latin American and the Caribbean Irrigated Rice (FLAR).

Rice research in LAC has emphasized growth, equity and the enhancement of the natural resource base. Technological progress, improved efficiency, huge production increases and important linkages with the rest of the economy have put rice as the highest priority for agricultural growth in most countries of LAC. Lowland rice has helped to meet food demands of the poor. New rice germplasm has helped to release pressure on fragile forest margins.

New varieties represent a pool of valuable new traits in an adapted genetic background, thus, forming a foundation upon which farmers can increase and stabilize yields. By improving production efficiency, these varieties generate cost savings for farmers, much of which was then passed on to consumers as lower prices.

By using pedigree information, researchers have estimated that the genetic diversity of most of LAC irrigated rice varieties is reaching its limits in terms of yield potential. Further yield enhancement requires the deployment of alternative genetic traits. Monitoring advances in diversification of the genetic base may benefit from the use of more precise methodologies than pedigree analysis. CIAT's major research emphasis in rice germplasm development therefore focuses on prebreeding activities with the aid of molecular markers, testing and adaptation of the new plant type from IRRI, crosses of commercial rice cultivars with wild rices and population improvement through recurrent selection. This is combined with conventional pedigree breeding. CIAT's role in the early next century is to complement breeding efforts of NARS, FLAR, and other partners in the region. Promising lines are made available to NARS and FLAR through the INGER network, and serve as parents for further regional breeding. The objective is to develop high yielding germplasm adapted to mainly irrigated conditions, tolerant to major diseases and insect pests, with good grain quality, and early to intermediate growth duration. To ensure good disease pressure during the selection process, "hot spot" sites are used.

Plant improvement efforts use a combination of biotechnological tools and classical plant breeding approaches. One objective is the identification and use of genes from wild germplasm for the improvement of yield and stress tolerance. To this end CIAT is developing and implementing a marker assisted breeding strategy (e.g. for yield, blast and RHBV resistance), that will lead to improved cultivars and simultaneously broaden the genetic base of cultivated rice. The rice project emphasizes the enhancement of populations, and has reduced considerably the production of fixed lines for direct release by NARS in the region. That task is assumed by FLAR and NARS. Thus the CIAT Rice Project intends to develop and enhance gene pools and populations for well-targeted traits to be used as a source of potential parents by regional breeding programs.

FLAR, bringing resources from private and public national organizations, has assumed the responsibility for the development of varieties and fixed lines, while both CIAT and FLAR conduct training.

### Improvement of Rice Germplasm for Latin America and the Caribbean

The goal of CIAT's Rice Project is to improve the nutritional and economic well-being of rice growers and low income consumers in LAC through sustainable increases in rice production and productivity. Several biotic and abiotic constraints affect rice production in LAC. A rough estimate of the value of production lost due to major diseases, weeds, soil problems, climatic stresses, and physiological constraints that could be addressed through research amounts to an estimated \$880 million per year. To reduce this impact, CIAT's Rice Project activities concentrate on four main outputs that would help to reduce the major constraints in LAC rice production: 1) enhancement or broadening of gene pools, 2) increased understanding of the physiological basis of rice traits, 3) the characterization of rice pests and the genetics of resistance, and 4) assist NARS to set research priorities and enhancing their research capacities.

Enhancing Gene Pools: The objective is to develop germplasm with higher yields and simultaneously broaden the genetic base of cultivated rice in LAC. A suitable breeding method used for this purpose is recurrent selection. In 1992, a CIRAD/CIAT collaborative project on rice improvement was established by introducing gene pools and populations segregating for a male-sterile recessive gene. Highlights of this project have been the identification of adapted fertile genotypes for use in breeding programs, recombination of the best selected genotypes with the introduced germplasm, recombination of the best locally adapted CIAT lines with the best adapted introduced germplasm, and resulting breeding lines were shared with several national programs for evaluation and selection.

Recurrent selection activities for irrigated rice are now being developed with most countries in LAC.

Identification of genes from wild germplasm for the improvement of yield and stress resistance includes the use of the wild species *Oryza rufipogon*, *O. glaberrima*, and *O. barthii* in genetic crosses with improved rice varieties. Transgressive segregation has been observed in several of the resulting lines, which yield between 5 and 25% higher than the commercial variety used in the respective crosses. This suggests that DNA introgressed from wild rices can increase yield in elite rice varieties.

Since 1985, CIAT has used anther culture (AC) as a tool to reduce generation time in rice. The use of AC has increased the availability of doubled haploids to fix desired traits, making this tool more cost-efficient for breeders, especially for southern latitudes. When compared to the traditional pedigree method, AC appears to be economically practical. CIAT is also using AC in backcrossed populations, to develop populations for molecular tagging of genes associated with cold tolerance, disease resistance, and tolerance to submergence. Our experience has shown that AC 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 tend to dominate in wide-cross populations when generations are advanced through selfing. AC is now a routine in several national programs of LAC, including to bridge wide crosses.

Molecular markers are used in breeding for rice blast resistance. Tagging and dissecting blast resistance genes in rice varieties that exhibit durable resistance is used for molecular markers such as RFLP, AFLP, RAPD, and microsatellites. Breeding populations analyzed include double haploid and recombinant inbred lines. Several useful markers (e.g. RAPD B10 linked to the blast resistance gene Pi-2; RFLP linked to blast resistance gene Pi-1) have been identified and are used in marker assisted selection for resistance to rice blast. Pyramiding of complementary resistance genes (Pi-1 and Pi-2) in a common background has demonstrated to be effective to control blast populations exhibiting great diversity and virulence. Our aim is to develop rice varieties with more durable and stable blast resistance than current varieties.

CIAT increased its research emphasis on grain quality. Most Latin American rice consumers prefer long, slender, translucent grain that cook dry and loose, and remain soft after cooling. Millers require a high percentage of head rice (percentage of whole grains after milling). We aim to better understand the genetic control of factors affecting grain quality. Routine analyses are conducted for grain length, white belly, gelatinization temperature, amylose content, and for milling quality.

About 40% of LAC's 6.7 million hectares of rice areas are in the freely drained (aerobic-soil) uplands, producing almost 20% of LAC's rice. Upland rice germplasm with higher yield is being developed for LAC. The first lines are now reaching the farm and promise an important breakthrough. The new upland varieties are shorter, use fertilizers more efficiently, do not lodge easily, and have better grain quality than traditional upland varieties.

Understanding the physiological basis of rice traits: This research concerns mainly the new plant type of IRRI. It seeks a plant type more competitive with weeds, and studies mechanisms for tolerance to low phosphorus and acid soils. Promising material of the new plant type constitutes a new source of germplasm that will broaden the genetic base and raise the yield ceiling. Selections have been made mainly for their large panicle size and strong culms.

Research on integrated weed control focuses on identifying germplasm with allelopathy and anaerobic vigor. The greatest expenditure in pesticides at the regional level is for herbicides. Red rice, which is the most troublesome weed in rice, cannot be controlled by rice herbicides. Weed control is greatly increased by permanent flooding until four to six weeks after seeding rice. New selections are made which possess the enzymatic activity necessary to grow under anaerobic conditions. Crosses are made to transfer tolerance to submergence into more adapted backgrounds.



Characterizing Rice Pests and Genetics of Resistance: Blast is one of the most severe diseases affecting rice production in LAC. The CIAT Rice Project has focused on the development of durable blast resistant varieties. Blast pathogen population studies using molecular markers and virulence analysis greatly increased efficiency in breeding, reduced costs, and had a positive implication on the sustainability of rice production. The molecular technique called MGR-DNA fingerprinting allows us to characterize populations of the blast pathogen and to identify resistance genes and gene combinations, resulting in lines that possess durable resistance. The highlights of this research include: understanding the dynamics of the blast pathogen over time and pathogen changes leading to resistance breakdown; identification of sources of resistance to develop rice lines combining complementary resistance genes that exclude compatible interactions with different blast pathogen populations (e.g. combination of blast resistance genes Pi-1 and Pi-2); and training of scientists in the use of new rice blast research technologies.

Evaluation and selection of resistant lines to blast is conducted under severe blast pressure in the experiment station of Santa Rosa in Villavicencio, Colombia. Most virulence factors present in Latin America are also present at this site, increasing the probability that a resistant line selected here will be resistant in other countries. To insure a uniform pressure for blast, "spreader rows" of susceptible commercial cultivars are planted in perpendicular rows several weeks prior to the test material. Spreader rows are composed of cultivars susceptible to the different genetic families of the blast pathogen present in Colombia. Blast samples are collected and analyzed for their genetic structure, virulence, and frequencies. Greenhouse inoculations are conducted to corroborate field reactions of parental lines or potential sources of resistance. Blast isolates exhibiting a wide spectrum of virulence and representing the entire genetic composition of the blast pathogen population in Colombia are used for the controlled inoculations.

Resistance against rice hoja blanca virus, RHBV, and its planthopper vector *Tagosodes oryzae* are essential traits for rice varieties in LAC. Diverse gene pools and breeding lines are evaluated for resistance to *T. oryzae* throughout the year under greenhouse conditions. Epidemics are cyclic and outbreaks 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 for RHBV resistance is to inoculate plants using a highly viruliferous colony of the insect containing more than 80% of active vectors. Maintaining insect colonies with high percentage of vectors is a routine but essential activity of the rice project. The planthopper can be genetically resistant to RHBV, and to maintain a viruliferous colony, one must continually select for susceptible planthoppers. The increase in percentage of resistant lines to RHBV and the planthopper vector is encouraging.

A novel source of RHBV resistance is being developed by the production of transgenic rice through nuclear protein (N) mediated cross protection and anti-sense RNA strategies by ballistic bombardment of rice tissue of the cultivar Cica 8. Several T1 transgenic rice lines have been identified with resistance to RHBV. These plants contain multiple copies of the N-gene plasmid construction, which carries the genes for resistance. The trait for resistance was still segregating at the T2 generation. Several plants showing stable resistance combined with good agronomic traits, fertile, and high yield potential have been selected. These are being crossed with commercial cultivars to study gene expression, and transfer of this gene into selected breeding materials.

Assisting in the development of regional research: CIAT's Rice Project evolved from the past breeding and agronomy program into a strategic focus on pre-breeding activities that complement the work of FLAR. Ten member countries in LAC plus CIAT, IRRI, and CIRAD assumed the financing and management of FLAR's research and training agenda. This effort is based on efficiency in research and cooperation with the regional research system. The basic research objectives of FLAR are: to provide access to and interchange of elite germplasm and information through INGER-LAC network; develop and characterize parents for use in temperate and tropical breeding programs; develop rice cultivars with stable resistance to the principal stresses in the region; select for grain quality and milling; and promote strategies of crop management for sustainable rice production.

In terms of breeding, FLAR develops mainly triple crosses. Crosses for temperate zones are advanced through double haploid breeding in cooperation with the Rice Project. Parental lines and breeding populations are characterized for morphological and physiological traits such as height, earliness, vegetative vigor, grain quality, and resistance to blast, brown spot, leaf scald, grain discoloration, hoja blanca, and *Tagosodes*. These are critical factors for new lines adapted to the various environmental conditions in Latin America.

Enhancement of the research capacities in the region are achieved through short courses and in-service training. The objectives of the training activities are: understanding of the different activities and developments in plant breeding and how to use them to solve local problems; promoting the interchange of ideas among participants; defining the flow of germplasm in the region; and standardizing methodologies of evaluation and selection of experimental lines.

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**Table 1. Rice varieties released in Latin America and the Caribbean, by origin, 1970-98.**

Region	Total	Local	IRRI	CIAT	Other	Contribution CIAT (%)
Caribbean	27	19	4	4	0	15
Cuba	14	11	2	0	1	0
Mexico	36	21	10	3	2	8
Central America	46	6	2	37	1	80
Tropical Brazil	57	31	2	18	6	32
Temperate Brazil	37	16	5	14	2	38
Andean countries	62	8	9	39	6	63
Southern Cone	16	11	0	3	2	19
Total	295	123	34	118	20	40

**Table 2. Percentage of modern semi-dwarf varieties (MSV) in production and in area, Latin America and the Caribbean (LAC).**

Production system	Percentage in production				Percentage in area			
	1966	1981	1989	1995	1966	1981	1989	1995
Irrigated	0.0	79.3	88.1	98.3	0.0	76.4	84.7	97.6
Rainfed	0.0	53.3	69.3	76.7	0.0	50.3	61.8	71.7
Subtotal wetlands	0.0	73.5	84.2	92.8	0.0	69.7	79.1	89.5
Mechanized upland	0.0	6.9	13.3	24.7	0.0	5.8	10.3	18.0
Traditional upland	0.0	30.0	30.0	30.0	0.0	26.0	28.2	31.2
Total MSV (%)	0.0	49.9	67.5	80.3	0.0	28.2	43.6	58.8

# SUSTAINABILITY OF RICE PRODUCTION IN EGYPT

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## ABSTRACT

Rice is one of the most important crops in Egypt; it occupies, annually, about 0.600 million hectares. Its production plays a significant role in the strategy to overcome food shortage and improvement of self sufficiency for local consumption and export. Because of the limited land available for cultivation in Egypt, the National Rice Research Program (NRRP) has aimed at increasing production and yield of rice in the last two decades by cultivating high-yielding short and long grain varieties. Egypt has been ranked first among the main rice producing countries with a yield average in 1980 of 5.71 ton/ha, reaching 8.56 ton/ha in 1997. This represents an increase of about 2.77 ton/ha or 47.7% throughout that time period. These achievements are due to the well organized Multi-Disciplinary Rice Research program and the efficiency of the vital role of the National Rice Campaign. Recently researchers have managed to cultivate short duration new varieties that are harvested 30 to 45 days earlier than the long-aged traditional varieties with high productivity of 12.0 ton/ha. Also the yield/day of the short duration varieties is higher than the long duration traditional varieties. This translates to saving 3.5 million m<sup>3</sup> of irrigation water which represents about 30% of the rice water consumption. The strategy of NRRP is to reduce the gap between rice productivity (13 ton/ha) and the national average (8.6 ton/ha) through sustainability factors, which are: government policy, improved technology, external support, and farmers' participation.

## INTRODUCTION

Sometime in 2005, a mother will give birth to a very significant child - our country's 75 millionth being. Egyptian populations will be on its way to the 100-million level, expected in 2025. Even if family planning programs cut the birth rate to two children per family, the 100 millionth person will arrive well before the year 2025.

The exploding population rate grimly dictates the Egyptian food requirements, especially for rice. A truly formidable challenge faces those involved in the National Rice Research Program struggle to produce more rice. Over the next decade, the Egyptian rice production must increase by at least 20% just to maintain current levels of consumption. Otherwise, chances are high for wide shortage and even hunger.

The challenge of increasing productivity is it must be achieved without using more land and water. The major thrust of rice scientists in the next decade must be to develop high-yielding varieties. These new varieties must possess other qualities, such as earliness, decreased water requirements, and more efficient use of land. These new varieties must be resistant to pests, diseases, and weeds (allelopathy) to prevent environmental pollution as a result of chemical use. These new varieties must tolerate adverse conditions such as salinity and drought. Finally, there must be excellent grain quality to minimize the loss during milling.

In order to achieve this goal, the National Rice Research Program in Egypt must explore new directions and apply the vast potential technologies such as developing an Egyptian hybrid rice and using biotechnology, as well as the traditional methods of research that have served well. Such accomplishments can have great significance for rice production where the more traditional research techniques have been notably underused.

## FACTS ON RICE IN EGYPT

1. Rice is the preferred food by most Egyptians. It contributes about 20% to the per capita cereal consumption.
2. Rice is an important export crop:

1970	1980	1987	1991	1992	1993	1994	1995	1996
400,000	25,000	70,000	200,00	177,00	133,00	300,00	350,00	253,000 (tons)

3. Rice occupies about 600 thousand hectares or about 22% of the cultivated area in Egypt during the summer season.
4. Rice consumes about 10 million m<sup>3</sup> or about 18% of the total resources.
5. Rice farming engages about 1 million families or 10% of Egypt's population.
6. The average annual value of rice during the period 1994-1997 amounted to about 1 billion LE, representing about 12% of the average annual value of plant production and about 8% of the total value of agricultural production.
7. The measures used to assist rice producers are limited. The floor price of \$215/ton for short grain Japonica rice varieties in 1997, while decreased to \$175 for long grain Indica rice varieties, and the impact on selling the long grains varieties not accepted for Egyptian consumption.

### RECENT DEVELOPMENTS IN RICE PRODUCTION IN EGYPT

Further increase in rice production from increased yield per unit area is needed. This can be achieved through varietal improvement, optimizing of cultural practices, as well as controlling weeds, diseases, and insects.

The objectives of the present rice production policy are:

- Self-sufficiency plus modest surplus for regional export.
- Sustain and improve rice production.
- Shorten the duration of the crop to decrease water requirement and increase crop identification.

Rice yields in Egypt have been among the world's highest due to fertile soil of the Nile Delta, high intensity of sunlight, few diseases and insect pests, warm weather and a good irrigation system. At 8.57 tons/ha, Egypt's national average yield of rice is more than double the world average of three tons/ha.

Recently the National Average Yield of rice increased from 5.71 tons/ha during the base period 1984-1986 to 7.09 tons/ha during the 1989-1991 period. It reached 7.71 tons/ha in 1993 and 8.56 tons/ha in 1997, which may be the world's highest (Table 1). Two of the seven rice growing governorates (Beheira and Gharbia) yielded about 9 tons/ha.

These high yields were achieved through:

- The release and spread of new short duration high-yielding varieties, Giza 175, Giza 176, Giza 181, Giza 177, and Giza 178, Sakha 101, and Sakha 102 (Table 2).
- Transfer of appropriate technology to the farming community to improve crop management, and
- Monitoring production constraints and farmers' problems during the season, and prompt follow-up by various agencies under the umbrella of the National Rice Campaign to alleviate the problems.

Studies conducted to determine yield potential, or "Yield Gap," showed yields of the demonstration fields, using the best package of recommended technology and improved high-yielding varieties, averaged 10.39 tons/ha from 1988-1997 (Table 3). Yields of the demonstration fields exceeded the national average yield by about 21%. 1997 results showed that potential yield ranged between 7.36-12.64 tons/ha (Table 4).

The gap between potential yield (about 13 tons/ha) at the demonstration fields and national average yield of about 8.56 tons/ha can be attributed to the following list of production constraints:

1. Soil salinity and alkalinity,
2. Inappropriate water management,
3. Large area (about 85%) planted to traditional japonica varieties,
4. Spread of blast disease,
5. Inappropriate pest management, and
6. High percentage of post-harvesting losses.

During the last 10 years (1987 to 1997), several goals were achieved in rice production in Egypt. These achievements included three main aspects: significant increase in yield per unit area, higher increase in rice production, and the highest increase recorded for area planted to rice.

With respect to the yields per unit area, the national average recorded 23% higher in rice productivity during this period than the previous period (1975 to 1986). The average yield was 5.71 t/ha during 1975 to 1986, while it reached 7.3 t/ha during 1987 to 1997, estimated as a 1.3 t/ha difference.

Regarding the whole production of paddy rice, the total production increased during this period from 2.4 million tons (Average 1975 to 1986) to 3.4 million tons (Average 1987 to 1997). The difference estimated as 1 million tons representing about 42% increase in rice production during the last 10 years over the previous period. This increase in rice production is attributed mainly to the improvement of the rice productivity per unit area, as well as the expansion of the rice area. Although rice area is limited by the Ministry of Irrigation to be 1 million feddan annually (0.4 million hectares), it exceeded this level during the last 10 years. This is mainly due to the farmers' preference to make more income by growing rice with its high productivity, high price, and less problems compared to cotton or maize.

The data reported have shown that a large increase in rice yields and production have been achieved during the last 10 years (1987 to 1997). This increase was mostly due to the development and release of very promising new varieties having high-yield potential, early maturity, and high resistance to blast disease. These varieties are widely spread in the farmers' fields because they are highly accepted by the farmers and consumers. These varieties are presented as follow:

**“Giza 181”**- The origin of this variety is IR 1626-203, as an International Rice Research Institute (IRRI) line introduced to Egypt from IRRI in the advanced generations. After a period of adaptation under Egyptian conditions, it proved to be a high yielding rice variety in Egypt. Besides its high-yield potential and high level of resistance to blast, it is also early maturing, non-lodging and has excellent grain and cooking qualities. It was released in 1987 and recommended for general cultivation as a new long-grain variety “Giza 180.”

**“Giza 175”**- The origin is a breeding line 1394-10-1 selected from the local top cross made between IRRI varieties and the local variety “Giza 14.” It has a particular advantage in that it combines both indica and japonica features, such as short grain japonica type and short stature, short growth duration, and high resistance to blast disease as the indica type. It was recommended for general cultivation in 1989, and was registered as a new variety “Giza 175” in 1991. It is acceptable to rice growers for its high yield potential and other agronomic characteristics, but it is less acceptable to the Egyptian consumers for its cooking and eating qualities and has now been replaced by “Giza 178.”

**“Giza 176”**- It is a breeding line 2175-5-6 selected from the local top cross “Calrose 76/Giza 172//GZ 242.” Giza 176 is a short grain japonica type released in 1989 and registered in 1991 as a new variety. It occupied more than 30% of the total rice area in 1991 due to its popularity with rice growers and consumers for its high yield potential and acceptable grain and cooking qualities. Also, it has short stature and medium growth duration. Giza 176 is still occupying a large area annually, although it became susceptible to blast disease.

During the last five years, more emphasis has been given to early maturity as the first priority in the breeding research, as well as the other important objectives - blast resistance, salinity tolerance, and grain quality. Early maturity is very important for stabilizing yield, economizing on water use and increasing farmers' income through cropping intensity at least one cut more of berseem clover, which is equal to one ton of paddy rice. These efforts have lead to the development and release of new early maturing varieties widely spread in the farmers' fields. The following is a brief description of these varieties.

**“Giza 177”**- A popular short-grain, japonica-type variety developed from the cross “Giza 171/Yomji No. 1//Pi No. 4” as a breeding line 4120-205. It was released in 1995 as the first early maturing rice variety. “Giza 177” has been a popular variety since it was released because it is about 30 to 40 days earlier than the old dominant late and tall varieties, Giza 171 and Giza 172. It has also yielded about 10% more than Giza 171 and Giza 172. Yield potential of Giza 177 is enhanced greatly by slightly decreasing transplanting spaces to compensate the low tillering ability. It is moderately awned under some specific conditions, has high resistance to blast disease, has high milling out turn, and has excellent cooking and eating qualities.

**“Giza 178”**- A pure line selection from the cross “Giza 175/Milyang 49” released in 1997, has a good tolerance to salinity and alkalinity and is widely adapted to the salt-affected soils. As one of its parents, Giza 178 is similar to Giza 175 in plant and grain type as a short-stature indica type and short-grain japonica type, but it is superior to Giza 175 in yield and grain quality, particularly in eating quality that is more acceptable to consumers than Giza 175. It has a very high-yielding potential and outyielded any other commercially grown variety at present. Giza 178 is also highly resistant to blast disease.

“**SAKHA 101**”- An intermediate maturing, short-grain, short-stature variety developed from the cross “Giza 176/Milyang 79.” It was released in 1997 to replace or to share Giza 176 in its area since it is more resistant to blast disease and higher in yield than Giza 176. It has superior grain quality with high milling out turn and translucent milled grains.

“**SAKHA 102**”- Sakha 102, like Giza 177, has shorter growth duration (102 days) than other Egyptian varieties, but its plant height is slightly taller than Giza 177. It is a pure line selection from the cross “GZ 4096/Giza 177” made at the Rice Research and Training Center (RRTC)-Sakha, and was released in 1997. It has high-yielding potential, is highly resistant to blast disease, and has excellent grain quality.

### **MULTI-DISCIPLINARY RICE RESEARCH PROGRAM**

With no land available for expansion and limited water resources, we must increase yield and productivity through a well-organized interdisciplinary team approach. Although Egypt is one of the few countries with an average yield of more than 8.5 tons/ha, twelve tons/ha is possible on experimental plots and demonstration fields.

An interdisciplinary research program was established in the early 1980s to narrow this gap and to encourage more farmers to adopt the improved production techniques. The program includes the following disciplines:

- Plant Breeding to develop new improved varieties with resistance to diseases and insect pests, early maturity and short stature,
- Seed Production to put pure seed of the new high-yielding varieties into farmers' hands,
- Agronomy including plant nutrition, water management, and cultural practices to maximize yields of the newly released varieties,
- Plant Protection against weeds, disease, insects and other pests,
- Mechanization, seeking small-scale implements that can be locally manufactured and maintained,
- Economics, keeping in mind that successful new technologies usually cut costs, and
- Extension to verify and transfer new technologies to the farmers.

#### **The Rice Research and Training Center**

In our efforts to strengthen rice research in Egypt, we have established the Rice Research and Training Center (RRTC) at Sakha, Kafr El-Sheikh Governorate with strong support of three main agencies:

1. Egyptian Ministry of Agriculture and Land Reclamation under Leadership of H.F. Dr. Yossef Wally,
2. Technical support of the International Rice Research Institute (IRRI) with whom we have been collaborating for about 20 years, and
3. Funding the Rice Research and Training Project through USAID from June 1980 to December 1986; through the National Agricultural Research Project (NARP) IRRI-contract, and recently through the Agricultural Technology Utilization and Training Project (ATUT).

The RRTC at Sakha, Kafr El-Sheikh Governorate was dedicated in January 1987. The Center has a full range of well-equipped research facilities, such as laboratories, glasshouses, screenhouses, library seed testing, cleaning and storage facilities, mechanical workshops, etc.

The RRTC houses about 90 Research Workers, 20 Senior Staff Members, 30 Research Assistants and some 50 Research Technicians.

In addition to Sakha, the RRTC has three testing stations at Gemmiza, Zarzoura and Sirw as well as twenty on-farm verification sites in the seven rice growing governorates (Kafr El-Sheikh, Dakahlia, Beheira, Sharkia, Gharbia, Damietta and Fayoum).

The Rice Research Program also employs about 150 Rice Production Advisors scattered in the seven rice growing governorates to help disseminate the improved technologies from Sakha to the various districts.

**Major Achievements:** Recently, intensive efforts have been devoted to improve rice production in Egypt. Consequently the National Average Yield of rice increased from 5.71 tons/ha (2.4 ton/fed.) in the base period 1984-1986 to 8.56 ton/ha (3.55 /fed.) in 1987 by 48.9% as a result of the following achievements:

- a) Release and spread of the short-stature, early-maturing, high-yielding varieties, Giza 181, Giza 175, Giza 176, Giza 177, Giza 178, Sakha 101, and Sakha 102,
- b) Improved nutrient management through efficient use of the nitrogen fertilizers and applying zinc sulfate to the rice nursery,
- c) Spread of chemical weed control from about 12% of the rice area in 1981 seasons to about 70% in 1997,
- d) Increased seed renewal rate from 50% in 1981 to about 70% in 1997 and improved seed quality, as well,
- e) Integrated blast management, including genetic resistance, cultural practices and chemical control, and
- f) Strengthened relationship among research, extension and rice growers through an efficient “National Rice Production Campaign.”

#### **ROLE OF ADAPTATION OF TECHNOLOGY IN SUSTAINING AGRICULTURAL PRODUCTION**

**Definitions:** Agricultural sustainability has been defined and described in many ways. All point to one dynamic concept - agricultural production needs should be catered to without degrading the natural resource base on which it depends. Sustainable agriculture is one that can evolve indefinitely toward greater human utility, greater efficiency of resource use and a balance with the environment that is favorable to mankind and other species. Further summarized, several common themes for development are as follows:

1. Food production must continue to increase to meet the demand of rapidly expanding populations (about 2% per year),
2. Total agricultural employment, as well as individual income from agriculture, must expand greatly,
3. Efficient use of capital, land and production inputs must increase sustainability, and
4. Production systems must be structured for the lowest possible use of pesticides.

#### **SUSTAINABILITY FACTORS**

A sustainable production system depends on four major factors with very close relationships among them. These factors are:

- Government policy
- Improved technology
- External support, and
- Farmers' participation

The following is a brief account for the role of each of these factors in establishing a sustainable production system:

##### **Government Policy:**

As mentioned earlier, political stability and commitment for all leaders starting from the President and Ministers down to village leaders, play an important role in sustaining agricultural production through establishment of various facilities, allocation of budget, and step-by-step follow-up of the implementation. Also there should be a national strategy with clear production goals and objectives for each commodity.

##### **Improved Technology:**

Research output could be in the form of information and technology related to production, economic, and social aspects. Production technology should be improved with more emphasis on:

- Breeding high-yielding, early-maturing varieties with resistance to the major diseases and insect pests,
- Crop management to maximize yield of the improved varieties and increase efficiency of irrigation water and fertilizers, and
- Integrated pest management to control weeds, diseases, and insects.

**External Support:**

Marketing inputs and outputs are very important factors affecting sustainable systems. The major inputs such as seed and fertilizers should be available to the farmers at the appropriate time. In addition, the floor and ceiling prices should be determined and announced at an early time before harvesting.

**Farmers' Participation:**

Farmers' participation and acceptance of the new technology is the aim of the technology adaptation program. It is the most important factor affecting sustainable systems.

**NATIONAL STRATEGY FOR RICE-PRODUCTION IMPROVEMENT TO YEAR 2000**

As mentioned earlier, during the Second Five-Year Development Plan (1987-1992) rice production increased from 2.4 million tons during the base period (1984-1986) to 3.4 million tons in 1991 by 42%. This large increase in rice production amounted to 49% due to high productivity (8.5 tons/ha) achieved in 1997.

To keep pace with the 2.7% annual population growth, while exporting about 500,000 tons annually, rice production has to be increased from the present 3.1 million tons (average of 1989-1991 period) to 4 million tons by the year 2000, i.e. about 30% of about 5% annually (Table 5).

To achieve this goal, a multi-pronged strategy has been developed as a part of the Third Five-Year Development Plan (1992-1997).

The strategy basically aims at increasing productivity per unit of land, water and labor, as well as increasing farmers' income from the rice based cropping system.

The strategy includes five major task areas as follows:

1. Support the Multi-disciplinary Rice Research Program to continue generation of improved technology with more emphasis on:

- a) Acceleration of the varietal improvement program to develop new japonica varieties with durable resistance to blast and early maturity.
- b) Intensify research on crop management to maximize productivity of the improved varieties and increase fertilizers and water use efficiencies,
- c) Intensify research on integrated pest management to control weeds, diseases, and insects with minimum use of pesticides, and
- d) Strengthen the collaboration with the International Rice Research Institute and other international organizations.

2. Sustain the Rice Production System through supporting the technology adaptation program at all phases (technology verification, technology demonstration, and mass-guidance) to motivate the rice farming community for active participation in the rice production program.

3. Improve productivity through expanding the area planted to the improved high-yielding, short-duration varieties and adoption of the new technologies for better crop management.

4. Identify the Low-Productive and Problem-Soils Districts. It has been reported that about 20% of the rice soils are salt affected and their productivity is about 40% less than normal soils.



5. Should enough irrigation water be available, it is suggested to expand the area under rice in the Nile Delta by 20% to become 1.2 million feddans (about one-half million hectares) in the year 2000.

**Table 1. Annual averages of rice area, production, and yield with responsive indicas during 1984-1997.**

Year	Area		Production		Yield (National average)	
	Hectares (1000)	Index	Tons (1000)	Index	Tons/ha	Index
1984-86*	420	100.00	2.400	100	5.71	100
1987	414	98.57	2.413	100	5.83	102
1988	360	85.71	2.182	91	6.06	106
1989	413	98.33	2.668	111	6.47	113
Average	396	94.29	2.421	101	6.12	107
1990	435	103.57	3.167	132	7.28	127
1991	454	108.10	3.411	142	7.51	132
1992	511	121.67	3.914	163	7.66	134
1993	538	128.09	4.140	173	7.71	135
1994	558	132.85	4.424	184	7.93	139
1995	587	139.90	4.821	200	8.20	143
1996	589	140.40	4.927	205	8.35	146
1997	672	160.00	5.752	240	8.56	150
Average	543	129.32	4.320	180	7.90	138

\* Base period 1984-1986

**Table 2. Yield and ancillary characters of the entries in the final yield trial-Sakha, 1994.**

No.	Entries	Yield	Days to heading	Plant height	Blast Reactions		Grain type	Milling %
					Leaf	Neck		
1	Giza 171 (Impr. CK)	7.68	118	135	5	S	Short	72
2	Giza 176 (Impr. CK)	9.88	103	95	2	R	Short	69
3	Giza 176 (Impr. CK)	10.02	110	100	6	S	Short	70
4	Giza 181 (Impr. CK)	9.76	113	95	2	R	Long	69
	Mean (Improved CKs)	9.89	109	97	-	-	-	70
5	GZ4120-205-2 (Giza 177)	9.10	89	96	3	R	Short	73
6	GZ 4255-6-3 (Giza 178)	10.22	96	95	2	R	Short	71
7	Sakha 101	10.67	103	90	2	R	Short	72
8	Sakha 102	10.00	90	105	2	R	Short	72
9	GZ 4255-6-32	10.23	95	95	2	R	Short	71
10	GZ 4462-10-2-2	8.41	90	95	2	R	Short	70
11	GZ 4681-5-2-1	9.26	91	95	2	R	Short	71
12	TKY 1014*	10.51	100	108	3	R	Short	73
	Mean (Tested entries)	9.81	94	97	-	-	-	72

\* INGER Entries

**Table 3. Egyptian rice yield gap in 1997.**

Year	Demonstration fields	National average
1988	9.90	6.07
1989	10.50	6.45
1990	10.43	7.29
1991	10.60	7.57
1992	10.64	7.67
1993	10.31	7.74
1994	10.45	7.93
1995	10.51	8.20
1996	10.29	8.35
1997	10.33	8.56
Mean	10.40	7.58

**Table 4. Yield of the demonstration fields-1997.**

No.	Variety	# Fields	Yield ton/ha		Remarks
			Average	Range	
1	Giza 176	1	8.81	8.81	Japanica
2	Giza 177	40	10.05	7.86-12.48	Japanica
3	Giza 178	17	11.07	8.43-12.64	Indica/Japonica
4	Sakha 101	10	10.62	7.36-11.67	Japanica
5	Sakha 102	6	9.90	8.12-11.90	Japanica
Total/average		74	10.33	7.36-12.64	

**Table 5. Rice production strategy 1992-2000.**

Period	Area		Yield		Production	
	1000 Fed.	%	T/Fed.	%	Mill Tons	%
1984-1986 (Base period)	1000	100	2.40	100	2.40	100
1989-1991	1033	103	3.00	125	3.10	130
1992-1997 “(2000)”	1200	120	3.50	146	4.00	167

## RICE GERMPLASM ENHANCEMENT AT IRRI

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Major advances have been made in increasing rice production worldwide as a result of large-scale adoption of modern high-yielding varieties and improved cultural practices. World rice production increased from 257 million tons in 1966 to 560 million tons in 1997. The present world population of 6 billion is likely to reach 8 billion by 2025. More than 70% of this increase will occur in Asia where rice is the staple food. It is estimated that we will have to produce 50% more rice to satisfy the growing demand for food. This increased demand will have to be met from less land, with less water, less labor and less chemicals. Thus, the challenges for the rice improvement are to develop varieties with higher yield potential, more durable resistance to diseases and insects, and higher levels of tolerance to abiotic stresses.

### **Increasing the Yield Potential of Rice**

Several approaches for increasing the yield potential of rice are being used. Some of these are:

- Population improvement
- Ideotype breeding
- Heterosis breeding
- Wide hybridization
- Genetic engineering
- Molecular Breeding

### **Population Improvement**

This is the time tested strategy for selecting crop cultivars with higher yield potential. It has two phases. The first phase involves the creation of variability and in the second phase involves selecting desirable individuals. Selection criteria involve growth duration, lodging resistance, disease and insect resistance, quality considerations, and adaptation to a specific environment. Selected genotypes are evaluated for yield and superior ones are released as varieties. It has been estimated that on average about 1% increase has occurred per year in the yield potential of rice over a 30-year period since the development of the first improved variety, IR8 (Peng, personal communication, 1999). Similarly, yield potential of wheats developed at the International Center for Wheat and Maize Improvement (CIMMYT) increased at the rate of 0.83% per year over the last 30 years (Fischer, 1994). The yields of the most crops where there is enough investment in research have been continuously improved and there is no reason why further increases cannot be attained.

### **Ideotype Breeding**

Ideotype breeding aimed at modifying the plant architecture is a time tested strategy to achieve increases in yield potential. Thus, selection for short-statured cereals such as wheat, rice, and sorghum resulted in doubling of yield potential. Yield potential is determined by the total dry matter or biomass and the harvest index (HI). Tall and traditional rices had HI of around 0.3 and total biomass of about 12 tons/ha. Thus, their maximum yield was about 4 tons. This biomass could not be increased by applications of nitrogenous fertilizers as the plants grew excessively, lodged badly, and the yield decreased instead of increasing. To increase the yield potential of tropical rice, it was necessary to improve the harvest index and increase nitrogen responsiveness by increasing lodging resistance. This was accomplished by reducing the plant height through incorporation of a recessive gene, *Sd-1* for short stature.

The first short-statured variety, IR8, developed at IRRI, also had a combination of other desirable traits such as profuse tillering, dark green and erect leaves for good canopy architecture, and sturdy stems. It responded to nitrogenous fertilizer much better and had a higher biomass (about 18 tons) and improved HI of 0.45. Its yield potential was 8 to 9 tons per hectare (Chandler, 1969).

To increase the yield potential of rice further, a new plant type was conceptualized in 1988 (IRRI, 1989). Modern semi-dwarf rices produce a large number of unproductive tillers and excessive leaf area, which cause mutual shading and reduce canopy photosynthesis and sink size, especially when they are grown under direct-seeded conditions. To increase the yield potential of these semi-dwarf rices, IRRI scientists proposed further modifications of plant architecture with the following characteristics:

- Low tillering capacity (3 to 4 tillers when direct-seeded)
- No unproductive tillers
- 200 to 250 grains per panicle
- Very sturdy stems
- Dark green, thick, and erect leaves
- Vigorous and deep root system

This proposed ideotype became the “New Plant Type” highlighted in IRRI’s strategic plan (IRRI, 1989) and the breeding effort to develop this germplasm became a major research project of IRRI. The goal is to increase the yield potential of rice to 12 tons or 25% higher than those of modern high-yielding, semi-dwarf varieties. Donors for developing the new plant type were identified and breeding work was initiated in 1989 (Khush, 1993; 1995). Numerous breeding lines with desired ideotype have been developed and are being evaluated in replicated yield trials. Several of the new plant type lines have outyielded IR72, which is one of the highest yielding semi-dwarf modern variety. Breeding efforts are now focused at improving the grain quality and incorporation of genes for disease and insect resistance.

### **Heterosis Breeding**

Yield improvement in maize has been associated with hybrid development. Yields of maize in the United States were basically unchanged from the mid-19th century until 1930 and accelerated greatly after the introduction of commercial double cross hybrids. The subsequent replacement of double cross hybrids by single cross hybrids in 1960 is associated with a second acceleration in maize yields. The average yield advantage of hybrids vs. cultivars is approximately 15% (Tollenaar, 1994).

Rice hybrids with a yield advantage of about 10 to 15% over best inbred varieties were introduced in China in the mid-1970s and are now planted to about 45% of the riceland in that country. Rice hybrids adapted to tropics have now been bred at IRRI and show similar yield advantage. Almost all the hybrid rices grown in China and India and those developed at IRRI are between indica varieties. The magnitude of heterosis depends upon the genetic diversity between the parents of hybrids. The greater the genetic difference, the higher the heterosis. During the past 30 years, the genetic differences among the improved indica rices have narrowed down due to massive international exchange of germplasm (Khush and Aquino, 1994). However, indica and japonica germplasm have remained distinct as there has been very little gene flow between these two varietal groups. As expected, hybrids between indica and japonica varieties show higher heterosis for yield than indica/indica hybrids (Yuan et al., 1989). IRRI’s new plant type program is based on tropical japonica germplasm. These improved tropical japonicas when used for producing hybrids with modern semi-dwarf indica varieties have higher level of heterosis.

The increased yield of tropical rice hybrid is due to the increase biomass, higher spikelet number and to some extent higher grain weight. The major constraints to the large scale adoption of hybrid varieties in the developing countries is the cost of hybrid seed that the farmer must buy for each crop of rice. To overcome this constraint, several institutes have active research programs to develop apomictic hybrids which will breed true and farmers will be able to use their crop harvest for planting next crop (Khush et al., 1994).

### **Wide Hybridization**

Crop gene pools are widened through hybridization of crop cultivars with wild species, weedy races as well as intraspecific crosses. Such gene pools are exploited for improving many traits including yield. Lawrence and Frey (1997b) reported that a quarter of the lines from BC<sub>2</sub>-BC<sub>4</sub> segregants from the *Avena sativa* x *Avena sterilis* matings were significantly higher in grain yield than the cultivated recurrent parent. Nine lines from this study when tested over years and sites, had agronomic traits similar to the recurrent parent and 10 to 29% higher grain yield. The higher yield potential of these interspecific derivatives was attributed to higher vegetative growth rates or early seedling vigor.

Tongil rices developed in Korea from indica-japonica crosses have 20% yield advantage over the best japonica parents (Ahn, 1995). More recently, Xiao et al. (1996) reported that some backcross derivatives from a cross between an *Oryza rufipogon* accession from Malaysia and cultivated rice, outyielded the recurrent parent by as much as 18%. They identified two QTL from wild species with major contribution to yield increase. These QTL are now being transferred to several modern semi-dwarf varieties.

### **Genetic Engineering**

Since protocols for transformation of rice are now well established (Shimamoto et al., 1989; Christou et al., 1991), it is now possible to introduce single alien genes that can selectively modify yielding determining processes. Starch biosynthesis plays a pivotal role in plant metabolism, both as a transient storage metabolite of leaf tissue and as an important energy and carbon reserve for sink organs such as seed, roots, tubers, and fruits. Thus, starch is the critical determinant of the sink

strength of developing sink organs as well as the source. Several enzymatic steps are involved in starch biosynthesis in plants. ADP-glucose pyrophosphorylase (ADPGPP) is a critical enzyme in regulating starch biosynthesis in plant tissues. Even in storage, organs with high levels of ADPGPP, its activity is still limiting. This limitation appears to be primarily at the level of allosteric regulation of the enzyme, at least in sink tissues. It should be possible to affect starch production in storage tissues positively by regulated expression of the gene encoding this enzyme (Kishore, 1994). Starch levels and dry matter accumulation were enhanced in potato tubers of plants transformed with *glgC*<sup>16</sup> gene from *E. coli* encoding ADPGPP (Stark et al., 1992). The transformed potato plants had tubers with higher dry matter and starch content, under both growth chamber as well as field conditions. The plants were similar in growth and development to nontransgenic controls. The nature of starch produced by the tubers containing *glgC*16 gene was similar to that of controls. This gene has been introduced into rice and transgenic plants are being evaluated.

In several crop species, incorporation of “stay green” trait or slower leaf senescence has been a major achievement of breeders in the past decades (Evans, 1993). In some genotypes with slower senescence (stay green), the rubisco degradation is slower which results in longer duration of canopy photosynthesis and higher yields. The onset of senescence is controlled by a complement of external and internal factors. Plant hormones such as ethylene and abscisic acid promote senescence while cytokinins are senescence antagonists. Therefore, overproduction of cytokinins can delay senescence. The *ipt* gene from *Agrobacterium tumefaciens* encoding an isopentenyl transferase (Akiyoshi et al., 1984) was fused with senescence-specific promoter, SAF12 (Gan and Amasino, 1995) and introduced in tobacco plants. The leaf and floral senescence in the transgenic plants was markedly delayed, biomass and seed yield was increased, but other aspects of plant growth and development were normal. This approach appears to have great potential in improving the crop yields through slowing the senescence and rubisco degradation and thus increasing canopy photosynthesis. The *ipt* gene has been introduced into rice and the transgenic plants are being evaluated.

### **Molecular Breeding**

Crop yields are polygenically inherited and are strongly influenced by environments. Therefore, determination of genotypic values from phenotypic expression is not precise and selection strategies must take into account low heritabilities. Breeders generally select for yield on the basis of plant type in early segregating generations and on the basis of yield trials when uniform breeding lines become available. Until now it has not been possible to select for individual quantitative trait loci (QTL) having positive effects on yield in segregating populations. Recent developments in the construction of saturated molecular maps of several crops based on RFLPs, RAPDs and microsatellites has made it possible to map individual QTLs for yield. Molecular genetic maps consisting of numerous markers covering the entire genome of rice have been prepared. Through linkage analysis of large segregating populations, polymorphic for molecular markers and QTLs for yield, location of the QTLs for yield, can be determined. Selection for yield can then be done via closely linked molecular markers. In a cross between parents differing in QTLs for yield, individuals having positive QTL alleles for yield from both parents can be selected. Tanksley et al. (1989) and Stuber (1989; 1992) have elaborated the theory behind the molecular basis of selection for QTLs. A project to enhance the yield potential of rice through molecular marker aided selection is underway at IRRI.

### **Breeding for Durable Resistance**

Full yield potential of modern rice varieties is not realized because of the toll taken by the attack of disease and insect organisms. It is estimated that diseases and insects cause yield losses of up to 25% annually. Genetic improvement to incorporate durable resistance to pests is the preferred strategy to minimize these losses. There is no cost to farmers and resistant cultivars are easily adopted and disseminated unlike “knowledge based” technologies. Also, concern for the environment has become an important public policy issue and pest management methods that minimize the use of crop protection chemicals are increasingly finding favor.

Diverse sources of resistance to major diseases and insects have been identified and rice varieties with multiple resistance to diseases and insects have been developed (Table 1). However, no sources of resistance to sheath blight are available and there is paucity of donors for resistance to virus diseases and stem borer. Recent breakthroughs in cellular and molecular biology have provided tools to develop more durably resistant cultivars and to overcome the problem of lack of donors for resistance.

### **Wide Hybridization for Disease and Insect Resistance**

Wild species of *Oryza* are a rich source of genes for resistance breeding. For example, none of the cultivated rice was found to be resistant to grassy stunt. *Oryza nivara*, a wild species closely related to cultivated rice was found to be resistant and the dominant gene for resistance was transferred to improved germplasm through backcrossing. Several resistant varieties were developed and are widely grown (Khush, 1977). When the genes are to be transferred from more distantly

related species, special techniques such as embryo rescue are employed to produce interspecific hybrids. Jena and Khush (1990) transferred genes for resistance to three biotypes of brown planthopper from *O. officinalis* to an elite breeding line. Multani et al. (1994) transferred genes for resistance to brown planthopper from *O. australiensis* to cultivated rice. Similarly genes for resistance to blast and bacterial blight have been transferred from *O. minuta* to improved rice germplasm (Brar and Khush, 1997).

### **Molecular Marker Assisted Breeding**

Numerous genes for disease and insect resistance, are repeatedly transferred from one varietal background to the other. Most genes behave in a dominant or recessive manner and require time consuming efforts to transfer. Sometimes the screening procedures are cumbersome and expensive and require large field space. If such genes can be tagged by tight linkage with DNA markers, time and money can be saved in transferring these genes from one varietal background to another. The presence or absence of the associated molecular marker indicates, at an early stage, the presence or absence of the desired target gene. A molecular marker very closely linked to the target gene can act as a “tag” which can be used for indirect selection of target gene.

Two of the most serious and widespread diseases in rice production are rice blast caused by the fungus *Pyricularia oryzae*, and bacterial blight caused by *Xanthomonas oryzae* pv. *Oryzae*. Development of durable resistance to these diseases is the focus of a coordinated effort at IRRI using molecular marker technology. Efforts to detect markers closely linked to bacterial blight resistance genes have taken advantage of the availability of 10 near isogenic lines having single genes for resistance. Segregating populations were used to confirm co-segregation between RFLP markers and the genes for resistance. Protocols for converting RFLP markers into PCR based markers and using the PCR markers in marker aided selection have been established (Zheng et al., 1995). The PCR markers were also used for pyramiding genes for resistance to bacterial blight. Thus *Xa4*, *xa5*, *xa13*, and *Xa21* were combined into same breeding line (Huang et al., 1997). The pyramided lines showed a wider spectrum and higher level of resistance than lines with only a single gene for resistance. MAS has also been employed for moving genes from pyramided lines into the new plant type (Sanchez et al., 1999).

### **Genetic Engineering**

Protocols for rice transformation have been developed which allow transfer of foreign genes from diverse biological systems into rice. Direct DNA transfer methods such as protoplast based (Datta et al., 1990) and biolistic (Christou et al., 1991) as well as *Agrobacterium*-mediated (Hiei et al., 1994) are being used for rice transformation. Major targets for rice improvement through transformation are disease and insect resistance.

### **Insect Resistance**

As early as 1987, genes coding for toxins from *Bacillus thuringiensis* (*Bt*) were transferred to tomato, tobacco and potato, where they provided protection against Lepidopteran insects. A major target for *Bt* deployment in transgenic rice is the yellow stem borer. This pest is widespread in Asia and causes substantial crop losses. Improved rice cultivars are either susceptible to the insect or have only partial resistance. Thus *Bt* transgenic rice has much appeal for controlling the yellow stem borer. Codon optimized *Bt* genes have been introduced into rice by several workers (Fujimoto et al., 1993; Wunn et al., 1996; Ghareyazie et al., 1997; Datta et al., 1997). *Bt* rices show excellent levels of resistance in the laboratory and transgenic greenhouse. When major symptoms of stem borer damage were reproduced in the greenhouse on control plants, *Bt* rice was free of the symptoms (Ghareyazie et al., 1997). To minimize the possible development of resistance breaking biotypes, attempts are being made to put the *Bt* gene under the control of tissue specific promoters, which direct the expression of the gene only in the pith or green tissues, the primary target of stem borer (Datta et al., 1997).

Besides *Bt* genes, other genes for insect resistance such as those for proteinase inhibitors,  $\alpha$ -amylase inhibitors and lectins are also beginning to receive more attention. Insects use diverse proteolytic or hydrolytic enzymes in their digestive gut for the digestion of food proteins and other food components. Plant derived proteinase inhibitors or  $\alpha$ -amylase inhibitors are of particular interest because these inhibitors are a part of the natural plant defense system against insect predation. Xu et al. (1996a) reported transgenic rice carrying cowpea trypsin inhibitor (Cpti) gene with enhanced resistance against striped stem borer and pink stem borer.

### **Disease Resistance**

Several fungal, viral and bacterial diseases cause serious yield losses. Sources of resistance to diseases such as blast, and bacterial blight have been identified within the cultivated rice and elite cultivars with resistance have been developed. However, sources of resistance to sheath blight are not available and only a few donors for resistance to rice viruses are known.

A highly successful strategy termed coat protein (CP) mediated protection has been employed against certain viral diseases such as tobacco mosaic virus in tobacco and tomato. A coat protein gene from rice strip virus was introduced into two japonica varieties by electroporation of protoplasts (Hayakawa et al., 1992). The resultant transgenic plants expressed CP at high level and exhibited a significant level of resistance to virus infection and this resistance inherited to the progenies. Sheath blight of rice caused by *Rhizoctonia solani*, which has a wide host range, causes serious yield losses. Sources of resistance to this disease are not available in the cultivated rice germplasm. Lin et al. (1995) introduced a 1.1 kb rice genomic DNA fragment containing a chitinase gene through PEG-mediated transformation. The presence of this gene in T<sub>0</sub> and T<sub>1</sub> transgenic plants was detected by southern blot analysis. Western blot analysis of transgenic plants and their progeny revealed the presence of two proteins that reacted with chitinase antibody. Progeny from the chitinase positive plants were tested for resistance to sheath blight pathogen. The degree of resistance correlated with the level of chitinase expression.

### Breeding for Abiotic Stress Tolerance

A series of abiotic stresses such as drought, excess water, mineral toxicities and deficiencies in soil, and unfavorable temperature affect rice productivity. The progress in developing crop cultivars for tolerance to abiotic stresses has been slow because of lack of knowledge of mechanisms of tolerance, poor understanding of inheritance of tolerance, low inheritability, and lack of efficient techniques for screening the germplasm and breeding materials. Nevertheless, rice cultivars with varying degrees of tolerance to abiotic stresses have been developed.

Rainfed rice is planted to about 40 million hectares worldwide. Vast areas suffer from drought at some stage of growth cycle. QTL for various component traits of drought tolerance have been mapped (Champoux et al., 1995) and the information is being used to develop improved cultivars with drought tolerance.

In some areas crop suffers from floods when it is submerged under water for up to 10 days. Rice cultivars cannot survive such prolonged submergence. A few rice cultivars have been identified which survive submergence for 10 to 12 days. Genetic analysis of one such cultivar, FR13A revealed that tolerance to submergence is controlled by one major gene and a few modifiers (Mohanty and Khush, 1985). Using FR13A as a donor, improved rice cultivars with tolerance to submergence have been developed (Mackill et al., 1993). The major gene for submergence, designated as *Sub1* is located on chromosome 9 (Nandi et al., 1997).

Millions of hectares of lands otherwise suitable for rice are not farmed or have low productivity due to high level of salinity. Rice varieties with excellent levels of tolerance to salinity have been identified (Ponnamperuma, 1984) and improved germplasm with salinity tolerance has been developed. A major gene for salinity tolerance designated as *Slt* has been located on chromosome 1 (Gregorio, 1997). Several QTL for salinity tolerance have also been identified.

Genetic engineering approaches hold great promise for developing rice with higher levels of tolerance to abiotic stresses. Accumulation of sugar alcohols is a widespread response that may protect the plants against environmental stress through osmoregulation. Mannitol is one of the sugar alcohols commonly found in plants. Tobacco plants lacking mannitol were transformed with a bacterial gene *mtlD*, encoding mannitol (Tarczynski et al., 1992). Mannitol concentrations exceeded 6 $\mu$  mol/g (fresh weight) in the leaves and in the roots of some transformants, whereas this sugar alcohol was not detected in these organs of control tobacco plants. Growth of plants from control and mannitol-containing lines in the absence and presence of added sodium chloride (NaCl) in culture solution was analyzed. Plants containing mannitol had an increased ability to tolerate salinity (Tarczynski et al., 1993). After 30 days of exposure under concentrations of 20 mM NaCl in culture solution, transformed plants increased in height a mean of 80% whereas control plants increased in height only a mean of 22% over the same interval. This approach is worth trying in rice.

Xu et al. (1996b) introduced *Hva1* gene from barley for production of embryogenesis abundant (LEA) proteins into rice plants. Transgenic plants had tolerance to salinity and water stress. It is believed that LEA proteins may play a protective role in plant cells under water stress conditions.

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**Table 1. Disease and insect reactions of IR varieties of Rice.**

Variety	Disease or insect reactions <sup>1</sup>							
	Blast	Bacterial blight	Tungro	Grassy stunt	Green leaf-hopper	Brown plant-hopper	Stem borer	Gall midge
IR5	MR	S	S	S	R	S	MR	S
IR8	S	S	S	S	R	S	S	S
IR20	MR	R	S	S	R	S	MR	S
IR22	S	R	S	S	S	S	S	S
IR24	S	S	S	S	R	S	S	S
IR26	MR	R	MR	S	R	R	MR	S
IR28	R	R	R	R	R	R	MR	S
IR32	MR	R	R	R	R	R	MR	R
IR36	MR	R	R	R	R	R	MR	R
IR38	MR	R	R	R	R	R	MR	R
IR42	MR	R	R	R	R	R	MR	R
IR46	MR	R	R	R	R	R	MR	R
IR50	S	R	R	R	R	R	S	-
IR54	MR	R	R	R	R	R	MR	-
IR58	MR	R	R	R	R	R	S	-
IR60	MR	R	R	R	R	R	MR	-
IR62	MR	R	R	R	R	R	MR	-
IR64	MR	R	R	R	R	R	MR	-
IR66	MR	R	R	R	R	R	MR	-
IR68	MR	R	R	R	R	R	MR	-
IR72	MR	R	R	R	R	R	MR	-
IR74	MR	MS	R	R	R	R	MR	-

<sup>1</sup> R = Resistant; MR = Moderately resistant; S = Susceptible; - not known

# RICE GERMPLASM ENHANCEMENT AT CNRRI

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## ABSTRACT

China is one of the original countries in Asia to cultivate rice (*O. sativa*). Rice is staple food in China, contributing nearly 40% of total calorie intake. In 1995, 30.74 million hectares were planted to rice, producing a total of 185.22 million tons of unmilled rice; the national average yield was 6.02 tons/ha. The history of rice cultivation in China dates back at least 7,000 years. Natural and human selection of rice adapted to varying ecological conditions and cropping systems have resulted in a broad array of cultivars. The diversity of both indigenous cultivars and wild rice is very rich. By the end of 1995, a total of 71,790 rice accessions were catalogued and 64,186 accessions were stored in the national genebank.

During the period of 1986 to 1995, the evaluation of rice germplasm resources was one of the China's priority programs. Major evaluation items were screening of rice germplasm for resistance to pests, and tolerance to drought and salinity stresses, as well as analysis of rice grain quality. At the same period, rice germplasm enhancement has been strengthened. In order to meet the requirements of rice breeders to develop new inbred rice and hybrids, the targets of rice enhancement focus on the screening and innovating the resources of the three lines of hybrids, wide compatibility genes, high multiple resistance to biotic and abiotic stresses, as well as superior grain quality, etc. The achievements of using enhancing germplasm to develop new hybrids, such as Xieyou 413 and Shanyou 413, have been realized.

## INTRODUCTION

### Rice Production and Germplasm Resources

China is one of the original countries in Asia to cultivate rice (*O. sativa*). Rice is staple food in China, contributing nearly 40% of total calorie intake. In 1995, 30.74 million hectares were planted to rice, producing a total of 185.22 million tons of unmilled rice; the national average yield was 6.02 tons/ha.

The history of rice cultivation in China dates back at least 7,000 years. Natural and human selection of rice adapted to varying ecological conditions and cropping systems have resulted in a broad array of cultivars. The diversity of both indigenous cultivars and wild rice is very rich (Cunshan, 1992; 1993).

Field survey and collecting of rice germplasm have been the force of conservation efforts in China. Four periods of exploration to assemble the national wide diversity of rice occurred: the 1930s, the mid-1950s, 1968 to 1982, and 1986 to 1990. On the basis of characterization of morphoagronomic traits of rice collections in both landraces and wild rice, a nationwide cataloging work was organized by the Institute of Crop Germplasm Resources of the Chinese Academy of Agricultural Sciences (CAAS) in Beijing in cooperation with the China National Rice Research Institute (CNRRI) and various provincial academies of agricultural sciences. As a result, a total of 71,790 rice accessions were catalogued and 64,186 accessions were stored in the national genebank at the end of 1995. (Table 1).

During the period of 1986 to 1995, the evaluation of rice germplasm resources was one of the China's priority programs. Major evaluation items were screening of rice germplasm for resistance to pests, and tolerance to drought and salinity stresses, as well as analysis of rice grain quality. As a result, many accessions were acquired. For example, 12 cultivars and promising lines from 8686 accessions of foreign introductions have multi-resistance to pests (Table 2).

### Rice Demand and Breeding Challenge

Based on per capita grain consumption of nearly 400 kg., including 150 kg. of unmilled rice, and the area planted to rice being stable at 32 million ha, the future population growth and grain supply and demand for grain and rice population can be estimated (Table 3) (Cunshan, 1997a; 1997b).

At present, the objective of rice breeding in China is emphasis on the following:

- To develop new cultivars and hybrids that are high or superior high yielding, which are adapted to different ecological and cropping systems.

- To develop new cultivars and hybrids with resistance or multiresistance to biotic and abiotic stresses, such as blast, bacterial leaf blight, bacterial sheath blight, brown planthopper, white-backed planthopper, cold, drought, and salinity, etc.
- To breed new cultivars and hybrids with good quality to meet the requirement of the market economy.

In general, rice breeders hope to breed the new conventional cultivars and hybrids which can combine high-yielding, multi-resistance and good quality, but, it is difficult to do so. In 1997, the Ministry of Agriculture of China approved the start of the superior high-yielding rice breeding program. Besides good quality and resistance to pests, the yield targets of superior high-yielding rice under favorable conditions are expected to meet the future requirement (Table 4) (Shaokai and Shihua, 1997).

Great achievements of breeding for novel semi-dwarf conventional rice cultivars and hybrid combinations have been made in the last four decades, so as a result the total rice production and yield per unit in China was also increased. However, rice production and yielding potential have stagnated since 1990 (Table 5) (Shaokai and Shihua, 1997). Rice breeders are facing the serious challenge of sustainable increase in rice yielding potential per unit.

### **Germplasm Enhancement and Achievements**

The use and benefits derived from conserved germplasm is the sole criterion for assessing the genetic conservation program for a crop. The use of rice germplasm includes both direct use in rice production and indirect use in rice breeding programs as parents. To prepare exotic germplasm for greater use by plant breeders, it is often necessary to break up the tight linkage between the desired genes and the undesirable alleles. Therefore, before use by plant breeders, the exotic germplasm (land races and wild species) must undergo a series of steps to facilitate its incorporation into agronomically acceptable backgrounds: conversion and enhancement or prebreeding (Te-Tzu, 1985).

At the CNRRI, in order to meet the requirements of rice breeders to develop new inbred rice and hybrids, the targets of rice germplasm enhancement focus on the screening and innovating the resources of the three lines of hybrids, wide compatibility genes, high and multiple resistance to biotic and abiotic stresses, as well as superior grain quality. The achievements of using enhanced germplasms to develop new improved varieties and hybrids have been made since the mid-1980s.

### **Rice Germplasm Enhancement for the Wide Compatibility Restorer Lines of Hybrids**

Heterosis use is a major component of the breeding strategies in rice. In recent years, rice breeders in China have given attention to shifting the yield frontiers by using the strong heterosis of intersubspecific hybrids. There were several approaches on developing intersubspecific hybrid (Lijun et al., 1994). First, the wide compatibility germplasm had to be found and its wide compatibility gene had to be incorporated in the hybrid parent to overcome F1 sterility. Then, the wide compatibility restorer (WCR) or CMS lines could be used in intersubspecific hybrid breeding program.

From 1989 to 1994, a total of 4082 rice accessions, representing a wide range of genetic diversity of *Oryza sativa* L., were screened for the presence of the wide compatibility gene. Accessions were identified as WCVs if they, when crossed indica and japonica, produced F1 plant fertility close to the mean fertility of testers but significantly higher than the mean F1 fertility between indica and japonica testers (Shaokai et al., 1990). After the WCVs were identified, the restorability of the WCVs were also evaluated based on the performance of their F1 hybrids with different cytoplasmic male sterility (CMS) lines. The accessions with both wide compatibility and restorability were classified as wide compatibility restorers (WCRs). Up to now, about 100 WCVs and several WCRs, such as Zhong 413, T2070, 9308 and others were obtained at CNRRI. For instance, in 1989, a wide compatibility line, 50465 bred by the Hunan Hybrid Rice Research Center was introduced from Wuhan University and planted in CNRRI's Hainan Island Experiment Station. From the segregating population, 22 plants were selected and crossed with Xieqingzao A (a semi-dwarf wild abortive CMS). The parent and F1 hybrids were planted in CNRRI experiment station in 1990. According their agronomic performance, heterosis, and resistance to pests, the promising male parent plant was confirmed and further purified, which was named as Zhong 413 (Fig. 1).

### **Zhong 413 Possesses Many Desirable Characteristics**

Zhong 413 has semi-dwarf plant type (110cm plant height), suitable growth duration (132-135 days), superior lodging resistance, relatively short and erect flag leaves, small tiller angle and strong tillering ability, large panicles (178 spikelets/panicles), medium-grain size (26.5g/1000 grains). But Zhong 413 has only medium-grain quality.

### **The Wide Compatibility of Zhong 413**

When used as the female parent to be crossed with indica testers (IR36, and Nanjing 11) and japonica testers (Akihikari from Japan and Balilla from Italy), Zhong 413 produced F1 hybrids with a normal range of fertility. The mean spikelet fertility of the F1 hybrids between Zhong 413 and these testers ranged from 53.2% to 80.8%, significantly higher than those

of inter-subspecific hybrid between indica and japonica testers, and is close to those of intraspecific hybrids (indica/indica or japonica/japonica) (Table 6). This result indicated that Zhong 413 is one of the WCVs.

### The Restorability to Different CMS of Zhong 413

To test the ability of Zhong 413 to commercially used CMS lines, six different CMS lines were crossed with Zhong 413 and F1 hybrids were tested as the single rice cropping and late-season rice cropping in Hangzhou area. The results indicated that Zhong 413 had a strong restoring ability for different CMS lines (Table 7).

### Development of Hybrid Combinations Using Zhong 413

From 1991 to 1995, a number of hybrid combinations were developed using Zhong 413 as the WCR male parent and the CMS lines Xieqingzao A, Shenshan 97 A, V20 A, D-Shan A, Maxie A as female parents. The hybrids were named as Xieyou 413, Shanyou 413, V-you 413, and Maxieyou 413. Of these, Xieyou 413 and Shanyou 413 were registered and released in Zhejiang and Sichuan provinces, respectively. Up to 1997, Xieyou 413 and Shanyou 413 were grown on about 60,000 ha in China. Their average yielding potential was about 10 to 15% higher than the most dominant commercial hybrids Shanyou 63 and Shanyou 10 (Table 8).

### Rice Germplasm Enhancement for Wide Compatibility CMS Sterile Line of Hybrids

Exploration of the strong heterosis of intersubspecific cross of rice has been hindered by the sterility problem in indica/japonica hybrids. Such incompatibility can be overcome by incorporation of the wide compatibility gene  $S_n^5$  into indica or japonica breeding lines. An alternative is to develop male sterile lines with gene  $S_n^5$ . The anthers of F1 hybrid between 02428, a japonica wide compatibility variety (WCV), and Peiai 64, an indica WCV were cultured. One double haploid (DH) with elite characters was selected from more than 80 DH lines and was used as recurrent parent to replace the nucleus of Zhenshan 97 A, a wild rice abortive (WA) type of cytoplasmic male sterile (CMS) line through successive backcross. A WA type cytoplasmic-nuclear interaction male sterile line O64A and its maintainer line, 064B, with wide compatibility, were successfully selected (Shengguang et al., 1997) (Fig. 2). The O64A is stable in male sterility with excellent flowering characters for outcrossing and strong wide compatibility for high combining ability in hybridization between subspecies, and maintainer line 064B has strong wide compatibility (Table 9). Among F1 hybrids between O64A and different restorer lines, the F1 hybrid of O64A and 9512, a japonica type restorer line, had large panicles with high fertility and great potential for use in rice production (Tables 10 and 11).

### Rice Germplasm Enhancement for the Resources of Resistance to Pest by Combining Conventional Breeding with Biotechnology

Brown planthopper, *Nilaparvata lugens*, is one of the more severe insect pests on susceptible rice varieties in large rice cropping areas in China. Resistance genes to BPH from numerous Chinese landraces have been identified. However, some collections of *Oryza officinalis* originating in China possess high level resistance to BPH. To meet the requirements of rice breeders to develop new varieties with resistance to BPH, studies on resistance to BPH of hybrid between *Oryza sativa* and *O. officinalis* have been done. In 1993, CNRRI workers made the interspecific hybrid between Zhong 86-44, a high yield and good quality improved *O. sativa* (AA genome) rice, and YD 1785, a distant wild species, *O. officinalis* (CC genome), originating from Guangxi Zhuangzu Autonomous Region. The F1 hybrids were achieved through embryo rescue technique and were completely male sterile. Upon the first backcrossing with the Zhong 86-44, BC1F1 plants were achieved through embryo rescue and were also completely male sterile. In the second backcross to Zhong 86-44, the BC2F1 plants through embryo rescue again were produced and looked remarkably similar to *O. sativa*, of which, six BC2F1 plants had resistance to BPH and normal fertility. By 1997, near-isogenic lines (NILs) of BC2F5 were obtained (Daibin et al., 1997) (Fig. 3). Study of these transgenic promising NILs will be continued, and the NILs will be used in rice resistance breeding programs.

### Rice germplasm enhancement for resistance to bacterial diseases

In addition, CNRRI staff using the cecropin *B* gene as a candidate for rice anti-bacterial genetic engineering developed transgenic rice to improve resistance to rice bacterial diseases. Using microprojectile bombardment, plasmid pSB1, a cecropin gene expression vector carrying a selectable marker gene (bar gene) and a chimerical cecropin *B* gene with the cecropin *B* gene coding region under the control of the 5' region of the rice actin 1 gene (Act 1), was transformed into immature embryos (12 days after pollination) of the commercial japonica type rice varieties Jinying 19 and Zhongbai 4. Three transgenic plants (JY-B1-3, JY-B1-4, and ZB-B1-1) were obtained after selection and regeneration. All transgenic rice plants were resistant to herbicide Basta, whereas all nontransformed plants were killed by 0.25% Basta spraying. PCR and southern analysis demonstrated that both cecropin *B* gene and bar gene have been integrated into the genome of the transformed rice.

According to the disease resistance tests, ZB-B1-1 showed high resistance to rice bacterial streak, *Xanthomonas campestris* pv. *oryzicola*, JY-B1-3 showed moderate resistance to both rice bacterial leaf blight, *Xanthomonas campestris* pv. *oryzae*, and rice bacterial streak (Danian et al., 1995).

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**Table 1. Rice germplasm accessions catalogued and stored from 1986 to 1995 in China**

Type	Accessions catalogued	Accessions stored
Traditional cultivars	50,530	46,941
Improved cultivars	4,085	3,285
Wild collections	6,944	5,243
Hybrid rice three lines*	1,605	1,042
Genetic markers	120	114
Foreign introductions	8,686	7,561
Total	71,970	64,186

\* Cytoplasmic male sterile, maintainer, and restorer lines.

**Table 2. Twelve foreign countries' cultivars with high resistance to three kinds of pests\***

Cultivar	Origin	Resistance score				
		BI	BB	BPH	WBPH	
IR 28	IRRI	0	1	1		
IR 29	IRRI	1	1	1		
IR 5853-162-1-2-3	IRRI		1	1	1	
IR 9115-40-1-3	IRRI		0	1	1	
IR 9782-111-2-1-2	IRRI		1		1	1
Horonamawee	Sri Lanka	1	1	0		
PDR 78-D5-D8-D4	Pakistan	1	1	1		
Bknbr 76025-10-8-1-2klg-1-1-1	Mexico	1	1	0		
ITA 233	Nigeria		1	1		1
IR 4227-24-3-1	IRRI		1		1	1
IRI 357	South Korea			1	0	0
RD 23	Thailand			0	0	0

\* BI-Leaf blast, BB-Bacterial blight, BPH-Brown plant hopper, WBPH-White backed plant hopper.

**Table 3. Population growth and grain supply and demand in China\***

Year	Population (billion)	Grain demand (million ton)	Grain per capita (kg/year)	Rice supply as 40% of grain (million tons)
1949	0.54	113	210	48.6
1995	1.2	466	386	183
2000	1.2	500	385	200
2010	1.4	550	390	220
2030	1.6	640	400	260

\* Figures of 1949 and 1995 were actual, other figures were forecasts.

**Table 4. Expected yield target of different superior high-yielding rice.**

Year	Conventional rice (t/ha)*				Hybrid rice (t/ha)*			Increasing (%)**
	EI(Y)	E&L(S)	SJ(Y)	SJ(N)	EI(Y)	SI&J	LI	
1990	6.75	7.50	7.50	8.25	7.50	8.25	7.50	0
2000	9.00	9.75	9.75	10.50	9.75	10.50	9.75	15
2005	10.50	11.25	11.25	12.00	11.25	12.00	11.25	30

\* Performance at two sites, 6.67 ha per site, successive two years

\*\* Yield trial at multiple sites, in comparison with check variety

EI(Y) - Early season indica rice (middle and lower reaches of Yangtze River)

E&L(S) - Early or late season indica (south China)

SJ(Y) - Single japonica rice (middle and lower reaches of Yangtze River)

SJ(N) - Single japonica rice (north China)

SI&J - Single indica or japonica rice

LI - Late season indica rice



**Table 5. Rice planting area, production, and yield from 1990-96 in China.**

Year	Area (Mha)	Production (Mt)	Yield (t/ha)
1990	33.064	191.74	5.805
1991	32.590	187.35	5.745
1992	32.090	186.22	5.803
1993	30.355	177.70	5.854
1994	30.172	175.93	5.830
1995	30.745	185.22	6.024

**Table 6. The F1 fertility in crosses between Zhong 413 and Testers.**

Cross	Type of cross	Fertility (%)	Cross	Type of cross	Fertility (%)
Zhong 413/IR 36	WCV*/indica	75.5	IR36/Najing 11	indica/indica	80.0
Zhong 413/Nanjing 11	WCV/indica	80.8	IR36/Akihikari	indica/japonica	32.2
Zhong 413/Akihikari	WCV/japonica	77.4	Akihikari/Balilla	japonica/japonica	67.4
Zhong 413/Balilla	WCV/japonica	53.2	Akihikari/IR36	japonica/indica	22.8

\* Wide Compatible Variety

**Table 7. The F1 fertility in crosses between Zhong 413 and different CMS lines (1991-1993).**

Cross of hybrids	Type of CMS*	Fertility (%)	
		Single season	Late season
Xieqingzao A/Zhong 413	SWA	75.2-93.1	74.0-89.8
V20 A/Zhong 413	WA	73.5-90.8	60.9-83.0
Zhenshan 97A/Zhong 413	WA	71.6-91.8	69.7-87.4
D- shan A/Zhong 413	DA	73.0-90.7	68.5-83.0
Maxie A/Zhong 413	MA	74.5-90.0	72.6-80.4
Shanyou 63 (CK)	WA	73.3-93.2	67.3-87.4

\* CMS - cytoplasmic male sterile; SWA - semi-dwarfism wild rice abortive; WA - wild rice abortive; DA - bring the CMS from the cross Dissi D52-37/Aigeonante; MA - bring the CMS from variety Maweizhan.

**Table 8. Average yield of the hybrids with wide compatibility restorer Zhong 413 (1993).**

Name	Cross	Single cropping		Late-season cropping	
		kg/ha	±CK*(%)	Kg/ha	±CK*(%)
Xieyou 413	XieqingzaoA/Zhong 413	8755	13.97	8097	13.40
Shanyou 413	Zhenshan97A/Zhong 413	8187	9.24	7886	15.59
Maxieyou 413	MaxieA/Zhong 413	7616	1.90	7988	5.68
V-you 413	V20A/Zhong 413	8353	5.13	8162	9.14

\* CK- Shanyou 63 (Zhenshan 97A/Minhui 63)

**Table 9. Wide compatibility of 064B.**

Female	Testers	Self fertility of F1 (%)
064 B	Nanjing 11 (indica type)	86.6
	IR 36 (indica type)	70.9
	Akihikari (japonica type)	88.0
	Balilla (japonica type)	93.1

**Table 10. Yield components in F1 hybrid between 064A and different restorer lines.**

Combinations	Productive panicles/ plant	Panicle length (cm)	Spikelets/ panicle	No. of grain/panicle	Seed set (%)	Days from sowing to heading (d)
064A / Milyang 46	15	24.3	183.3	158.4	86.4	87
Shanyou 46 (CK 1)	17	23.3	134.1	108.5	80.9	89
064A / Minghui 63	12	26.4	192.4	155.7	80.9	88
Shanyou 63 (CK2)	11	26.3	176.9	142.4	80.5	93
064A / 9308	14	26.7	206.9	179.6	86.8	91
Xieyou 9308 (CK3)	12	25.8	188.6	164.1	87.0	96

**Table 11. Yield and its components in 064A / 9512 and control.**

Combination	No. of panicle/ha	No. of spikelets/ panicle	Seed setting (%)	1000-grain weight (g)	Calculated yield (ton/ha)	Harvested yield (ton/ha)
064A / 9512	2.06 x 10 <sup>6</sup>	220.6	84.6	28.1	10.28	10.18
Shanyou 10 (CK)	2.79x 10 <sup>6</sup>	140.8	83.7	8.2	9.27	8.50

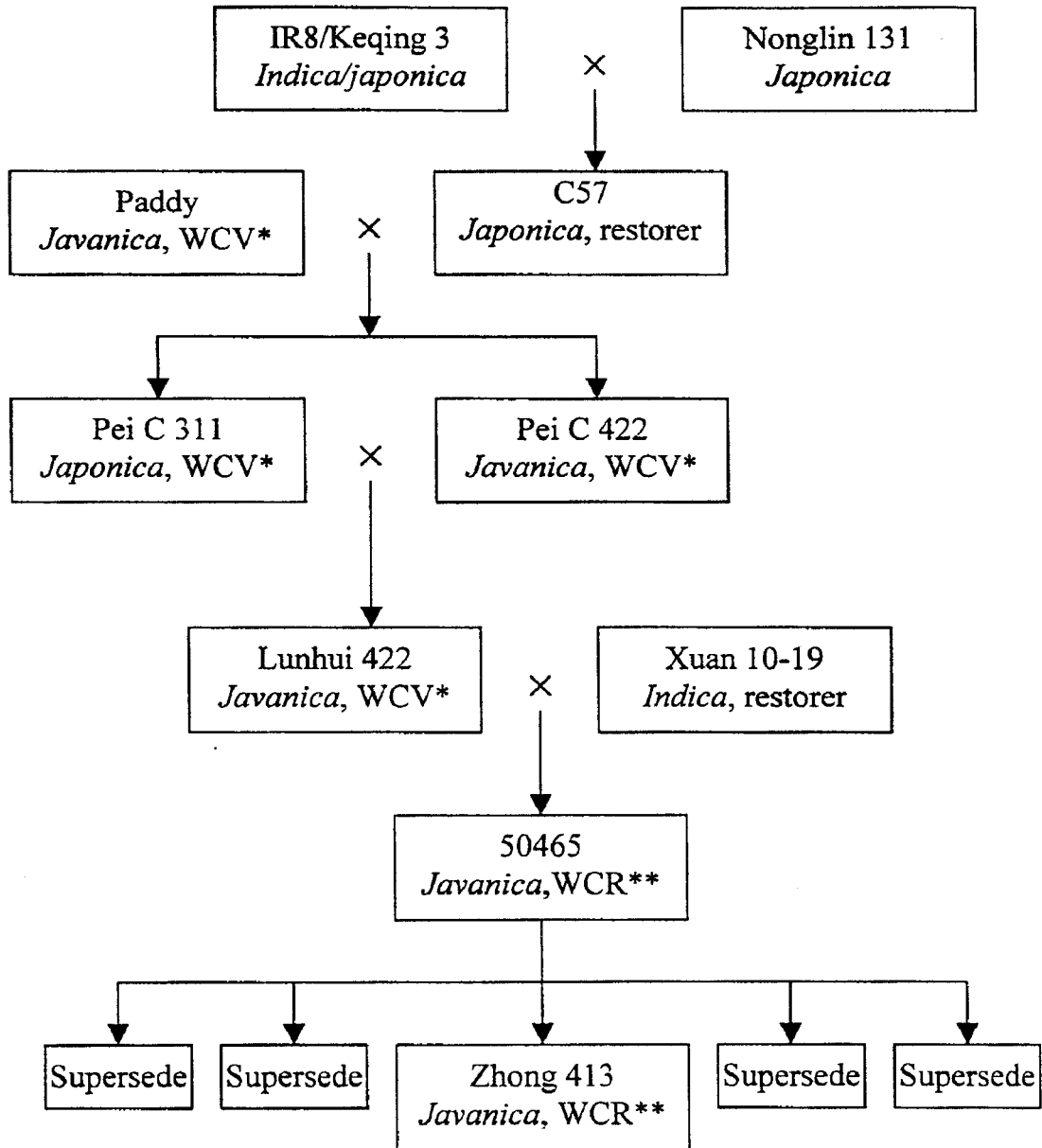


Fig. 1. Pedigree of wide compatibility restorer (WCR\*\*).

\* WCV: wide compatibility variety.

\*\* WCR: wide compatibility restorer.

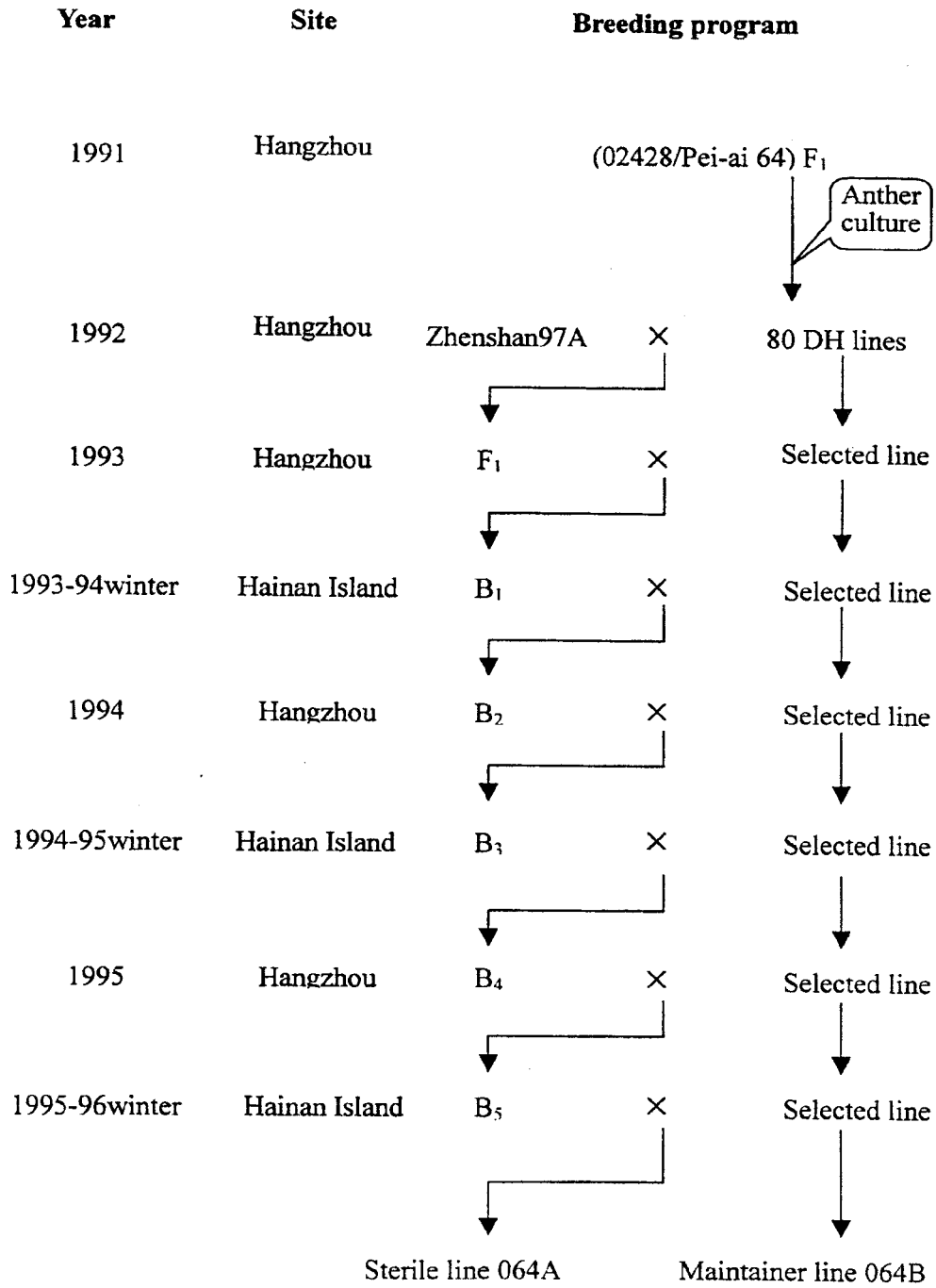


Fig. 2. Breeding program of 064A and 064B.

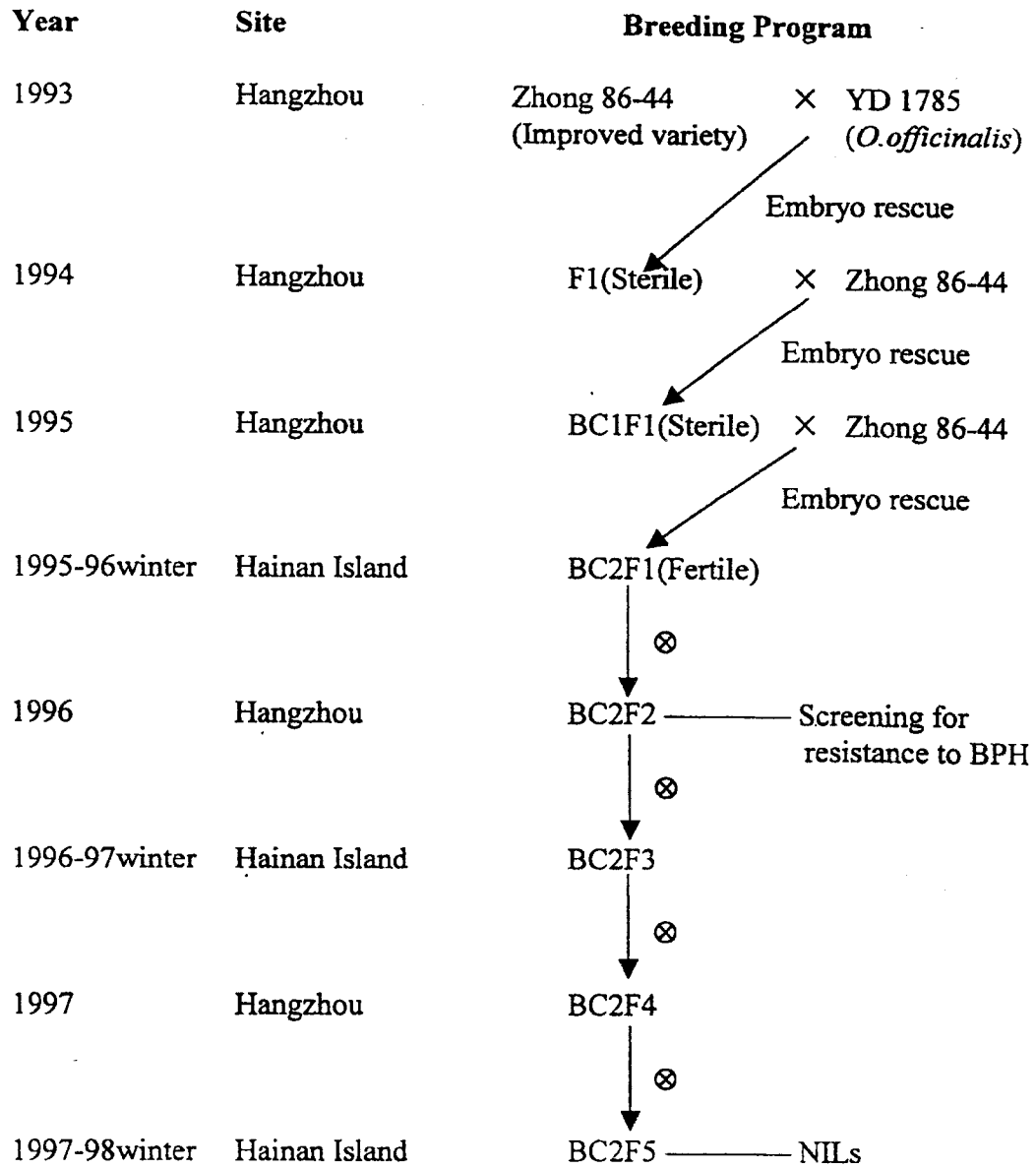


Fig. 3. Pedigree of Zhong 86-44/YD 1785.

# GERMPLASM EVALUATION AND ENHANCEMENT FOR DISEASE RESISTANCE

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## ABSTRACT

Germplasm evaluation and use for rice disease control is an ongoing process driven by genetic adaptations of the pathogen and by the frequent introduction of new cultivars to meet consumer and economic requirements. Disease resistance will remain the most effective and economical means for effective control of the many major and minor rice diseases found in all world production areas. The disease control strategy of using resistant cultivars depends completely upon germplasm evaluation and enhancement through traditional and the new molecular techniques.

## INTRODUCTION

Diseases are very often the major constraint which limits development and use of high-yielding rice cultivars. Traditional disease control strategies use resistant cultivars, cultural practices, and pesticide applications to control diseases. Cultivar resistance is the least expensive, most reliable and desirable means of disease control. Germplasm collection, evaluation, and enhancement for cultivar disease resistance is an integral component of the management tools needed to eliminate or reduce disease production constraints in rice.

In U.S. rice production areas, rice sheath blight, caused by *Thanatephorus cucumeris* (Frank) Donk, and rice blast, caused by *Magnaporthe grisea* (Hebert) Barr, occur with sufficient frequency and severity to be considered the most important yield limiting rice diseases. As a result, substantial resources are dedicated towards their control. Other diseases such as stem rot (*Magnaporthe salvinii* [Catt.] Krause & Webster), aggregate sheath blight (*Rhizoctonia oryzae-sativae* [Sawada] Mordue), brown spot (*Cochiobolus miyabeanus* [Ito & Kur.] Drech.) and kernel smut *Tilletia* (*Neovossia horrida*) *barclayana* ([Tak.] Padwick & Kahn) occur in susceptible cultivars and may cause significant losses but receive less emphasis in cultivar development programs. Several much less aggressive (minor) diseases are also common but cause only very limited losses. However, the minor diseases may suddenly become widespread with sufficiently increased severity to incite concern and cause a reevaluation of disease control priorities. For example, false smut caused by *Ustilagoideia virens* (Cooke) Takan is a minor disease which occurred very sporadically in production areas along the U.S. Gulf Coast. False smut was first observed in a few Arkansas production fields during 1997. During the 1998 growing season, false smut became widespread over a significant portion of the Arkansas rice production areas and is causing concern in the rice industry.

Current U.S. rice disease research programs have the overall objective of controlling yield limiting diseases. While all available disease control strategies are used, the discovery, use and maintenance of disease resistance genes provide the cornerstone for research programs that continually develop new high-yielding cultivars. Successful use of disease resistance requires an intimate knowledge of the pathogen, the available resistance genes, and their interaction in the various production areas.

Discovery and use of resistance is relatively straightforward with the rice sheath blight disease. Sources of major gene resistance for sheath blight are unknown, but several minor genes provide acceptable levels of field tolerance. Since the use of tolerance seldom favors virulence changes within the pathogen, tolerant cultivars are affected only by environmental variation and grower practices. Traditional breeding techniques, especially recurrent selection, have been successfully used to pool available resistance genes into sheath blight tolerant cultivars.

Rice blast is a much more complex disease. A highly variable pathogen, *M. grisea*, interacts differentially with multiple major and minor host resistance genes under equally variable environmental conditions. The pathogen is well known for its genetic variability. Numerous pathogenic races have been defined using phenotypic reactions on differential hosts. Fortunately, genetic variation of the *M. grisea* population within the United States is limited with only about 25 races having been described. Predominating races, typically only 5 to 10, tend to be localized within a production region with race prevalence

being determined by the acreage planted to specific susceptible cultivars. Major pathogen population shifts or virulence adaptations are usually detected within two to three years after a new cultivar is released for use by producers. In many instances, however, single major gene resistance provides effective blast control for several years.

Much of the world *M. grisea* population has been characterized using the MGR586 probe with isolates being classified according to DNA fingerprint groups or “lineages” (Zeigler et al., 1995). Phenotypic virulence correspond with the lineages with a few notable exceptions. Distinct vegetative compatible groups (VCG) based upon hyphae fusion between *M. grisea* isolates are also being used to study genetic variability within the population (Correll et al., 1998a; 1998b). Results indicate VCGs correspond with specific MGR-586 fingerprint lineages and will be helpful in further characterizing the population structure of this pathogen.

Several major and minor resistance genes for rice blast have been described within *Oryza sativa* L. As previously indicated, the causal organism, *M. grisea*, is well known for its genetic instability and often overcomes resistance in host plants. Regardless, plant resistance remains the most effective and economical control for the rice blast disease. Major resistance genes are more easily manipulated and provides effective control until a new virulent pathotype develops or an existing minor race increases in prevalence. Individual major genes or gene combinations have been used for several years in the United States without being overwhelmed by the blast pathogen (Lee, 1994; Marchetti, 1994). For example, the cultivar ‘Starbonnet,’ released in 1967 (Johnston et al., 1968), was only rarely damaged by blast race IG1 and was widely grown until replaced during 1984-1985 by much shorter-season, higher-yielding cultivars ‘Newbonnet’ (Johnston et al., 1984) and ‘Lemont’ (Bollich et al., 1985). Conversely, ‘Newbonnet’ was rapidly accepted and widely grown but proved to be highly susceptible to races IB-49 and IC-17 with substantial yield losses to blast occurring during the late 1980s. Some races virulent to newly released cultivars arise within the native pathogen population but do not incite significant disease. This is the case with the cultivar ‘Katy’ (Moldenhauer et al., 1990) released to growers in 1989 and its progeny ‘Kaybonnet’ (Gravois et al., 1995) and ‘Drew’ (Moldenhauer et al., 1998) which were released to growers in 1994 and 1995, respectively. The virulent race IE-1K detected on Katy (Marchetti, unpublished 1993), evolved either from within the native pathogen population as the result of natural selection pressure or from a spontaneous mutation for virulence. Subsequently, race IE-1K was immediately recovered from a few individual Kaybonnet plants in production fields during 1994 and from Drew in drought stressed growing conditions. Fortunately, race IE-1K is rarely found and has not caused damage to either of these cultivars presumably because growing conditions have been generally unfavorable for blast development or because the new race lacks the necessary virulence characteristics to incite blast epidemics. The pathogen population will be closely monitored in the future with the expectation that a significant combined production acreage will be planted to Kaybonnet, Drew, and other new releases using Katy as a donor parent for blast resistance genes.

The combined action of minor resistance genes confer blast “field resistance” where the plant is susceptible but pathogen development, and thus yield loss, is very limited. High-yielding, field-resistant cultivars are used with a certain degree of risk. Lemont, which is also susceptible to races IB-49 and IC-17, possesses field resistance and is still grown in limited acreage some 15 years after its release. The cultivar ‘Cypress’ (Linscombe et al., 1993), has resistance genes for some of the older U.S. blast races but is susceptible to prevalent blast races in the greenhouse where test conditions favor disease. However, Cypress is grown over a large production acreage in the southern United States without being affected by rice blast. The more susceptible, very high yielding cultivar ‘LaGrue’ (Moldenhauer et al., 1994) is grown without significant yield losses in localized production areas under optimum field conditions and cultural practices. Because pathogen adaptation to multiple gene resistance appears to be restricted, cultivars having very high field resistance are much more stable. However, it is difficult to develop field resistant cultivars because the presence of major gene resistance can obscure field resistance due to the lack of necessary techniques that quickly identify acceptable field resistance in breeding lines.

In the United States, the rice blast host-pathogen interaction is greatly impacted by research programs, which by necessity place a very high priority on developing new cultivars delivering substantially superior yield and agronomic characteristics and also meeting rigid consumer requirements. A constant search for and subsequent adaptation of, new germplasm sources provides these improved agronomic characteristics. Blast control achieved in existing pathogen-host balances is placed at risk and often compromised by new cultivars being released to achieve higher priority breeding objectives. Any increased disease liabilities in the new cultivar is considered to be an undesirable characteristic, which can be improved upon while immediate benefits are realized from growing the new cultivar.

To supply the constant demand for new disease resistance genes, U.S. rice disease control programs have used the traditional approach of evaluating potential *Oryza sativa* L. resistance sources in existing and introduced germplasm pools. Newly discovered resistance sources are promptly defined and used in ongoing breeding programs. The major blast resistance gene Pi-ta<sup>2</sup> in Katy (Moldenhauer et al., 1992) originated from Tetep (PI 280682) which was brought into the United States as an introduction from Vietnam. The international blast differential cultivar ‘Raminad Strain 3’ (PI 231128) has successfully withstood all challenges by U.S. pathotypes for many years (Marchetti, personal communication, 1994). Blast resistance genes in Raminad Strain 3 are now being transferred into plant types adapted for the southern U.S. rice produc-

tion areas. The ongoing evaluation of new rice germplasm having high yield potential and other desirable characteristics is providing new resistance genes that are more easily assimilated into U.S. rice production.

Since wild relatives are known to serve as an important source of disease resistance in related crop species, the U.S. rice disease programs also are investigating different *Oryza* species for resistance to rice blast, rice sheath blight, stem rot, and other rice diseases. Although improved resistance to rice diseases such as stem rot have been improved from this germplasm (Rutger et al., 1987; Tseng and Oster, 1994), the movement of blast resistance genes from related *Oryza* species into U.S. germplasm is only beginning (McClung et al., 1998). While the hope is to discover major genes providing broad resistance spectra to most lineages of blast, results to date suggest resistance in the *Oryza* species is limited to genes resistant to only a few races and/or lineages and, on the whole, may be as diverse and subject to pathogen variation as those in *O. sativa* (Eizenga and Lee, 1998; McClung et al., 1998).

Traditional breeding techniques based upon selection for phenotype expression or traits require significant development effort and time between discovery and use. To overcome this, new molecular techniques not affected by interaction with other genes or the environment are being used to aid selection in cultivar development. Disease resistance genes are being cloned and moved into susceptible cultivars via transformation. In addition, techniques are being developed to detect and define specific major resistance genes and/or quantitative resistance characteristics without the complications associated with phenotypic expression. Unfortunately, much of the molecular research is very expensive and to date has not been extensively used in the U.S. cultivar development programs.

Despite current drawbacks, molecular technology is gradually being used in the evaluation and enhancement processes. Lineage exclusion, the practice of selecting parents and progeny based upon their being resistant to select blast isolates representing all MGR-DNA lineages in the production area rather than their being resistant to specific pathogen isolates and/or pathotypes, has gained acceptance as a cultivar development tool (Gibbons et al., 1998). Lineage exclusion, however, has limited application in narrow genetic base pathogen populations. In these situations, the methodology of traditional selection provides a form of lineage exclusion where parents and lines in greenhouse and field tests are screened for reaction to predominating virulent blast pathotypes representing evaluation to contemporary lineages. As lines advance through the evaluation system, they are further screened with older pathotypes in the pathogen collection representing the remainder of lineages present within the production area. Lines highly susceptible to the older pathotypes are released for grower use only if very substantial agronomic advances are being realized.

The most immediate and promising application of molecular techniques is through marker assisted selection (MAS) techniques where molecular markers aid in locating specific DNA patterns and/or detection of major resistance genes in parents and progeny. One or more major genes, such as those in Katy, confer resistance to prevalent blast races and effectively mask phenotypic expression of, and thus selection for, other major and minor resistance genes present. Masking greatly inhibits efforts to pyramid multiple resistance genes and reduce the probability of pathogen adaptation to resistance genes present in new cultivars. Although multiple major genes have been pyramided using traditional breeding techniques as in 'Jefferson' which has Pi-k<sup>h</sup>, pi-d and Pi-z (McClung et al., 1997), the pyramiding process will be much simpler using MAS techniques as achieved with genes Pi-1(t), Pi-2(t) and Pi-4(t) (Mew et al., 1994).

MAS requires information about the location of the marker genes relative to specific resistance genes. To date, molecular investigations for blast resistance in U.S. germplasm are mostly limited to those on the cross 'Lemont'/'Teqing' where the location of major resistance genes and QTLs for contemporary pathotypes have been and are being mapped (Pinson et al., 1998a; 1998b). One very interesting technique used in the Lemont/Teqing population involves searching published information on the rice genome for simple sequence repeats (SSRs) (Conaway et al., 1998). Techniques developed to detect the SSRs location relative to specific blast resistance genes are being applied with a 85 to 90% success rate in a traditional rice breeding program (McClung, personal communication, 1998).

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# CROP GERMPLASM ENHANCEMENT THROUGH MUTATION TECHNIQUES

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## ABSTRACT

Following the recent trend in the release of crop mutant varieties in some countries, it is expected that the number of officially released mutant varieties listed in the FAO/IAEA Mutant Varieties Database will exceed 2000 entries by the year 2000. In December 1997, 1847 mutant varieties of 164 economic plant species were listed in the database. These varieties were released in more than 50 countries, but more than 1100 of them were in just six countries: China P.R., India, USSR and Russia, The Netherlands, Japan, and the United States. Recent developments in *in vitro* culture methods, improvement of large-scale selection techniques, and increasing possibilities for molecular marker application in breeding makes induced mutations a favored tool in crop improvement programs.

The spectrum of available mutation techniques has also significantly increased. Important practical results were achieved through the use of chronic irradiation from gamma-field or by irradiation with heavy ion beam. Somaclonal variation, generated through *in vitro* culture has proven to be useful for creating variation in many characters, especially the ones that can be selected under *in vitro* culture conditions. Classical insertional mutagenesis, and more recently retrotransposons, have become almost irreplaceable tools in generating and tagging of new mutations. All these mutagenic agents can be used to produce "morphological mutants" necessary in dissecting plant structure and function and their control. Mutational analysis of the most important characters determining plant productivity, though initiated in only a few species, should be considered for the most important crops as a prerequisite for functional genomics. Contrary to the vast amount of published reports on the recovery of various mutants, only a small proportion of them are kept in germplasm collections. Maize, Arabidopsis, and to some extent barley and tomato are the only positive examples of mutant germplasm conservation. In all these collections, the number of mutants with described and characterized mutations of genes responsible for plant productivity or for other agronomically important and desirable characters for breeding is exceptionally low. Keeping in mind the small size of the rice genome, it seems more realistic to initiate large-scale mutational work especially in this crop. The synteny of cereal genomes can help in the use of mutated genes in other cereals.

The traditional mission of mutation technology - development of new and desired variation for breeding programs - has been recently significantly widened. Induced mutations can play an important role in the conservation and preservation of crop biodiversity. Induced mutations and related technologies are important not only in extending genetic diversity of major crops but also are an important additional source of biodiversity enhancement of neglected and local crops.

## INTRODUCTION

Following the recent trend in the release of crop mutant varieties in some countries, it is expected that the number of officially released mutant varieties listed in the FAO/IAEA Mutant Varieties Database will exceed 2000 entries by the year 2000. In December 1997, 1847 mutant varieties of 164 economic plant species were listed in the database. These varieties were released in more than 50 countries, but more than 1100 of them were in just six countries: China P.R., India, former USSR and Russia, The Netherlands, Japan, and the United States. Recent developments in *in vitro* culture methods, improvement of large-scale selection techniques, and increasing possibilities for molecular marker application in breeding make induced mutations a favored tool in crop improvement programs.

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The traditional mission of mutation technology - development of new and desired variation for breeding programs - has recently been significantly widened. Induced mutations can play an important role in the conservation and preservation of crop biodiversity. Induced mutations and related technologies are important not only for extending genetic diversity of major crops but also are an important additional source of biodiversity enhancement of neglected and local crops.

### Mutational Analysis of Plant Structure and Function

Mutational analysis of the most important characters determining plant productivity, though initiated in only a few species, should be considered as a prerequisite for functional genomics for the most important crops. Contrary to the vast number of published reports on the recovery of various crop mutants, only a small proportion of them are maintained in germplasm collections. Maize, and to some extent barley and tomato, are the only positive examples of crop mutant germplasm conservation. In all these collections, the number of mutants with described and characterized mutations of genes responsible for plant productivity or for other agronomically important and desirable characters for breeding is exceptionally low.

“Phenotype gap” was first defined by Brown and Peters (1996) in relation to the genomics of mouse, a model species for dissection of basic pathophysiological mechanisms. According to the authors, many mouse mutations are extremely valuable for the investigation of human diseases and for identification of the critical genes involved in human pathologies. The concept of the phenotype gap can easily be extended to genetic investigation of plant species as a basic component of the mutational analysis of any plant species. The phenotype gap will reflect the gulf between available mutant resources and the full range of phenotypes of an investigated plant species. Brown and Peters (1996) reported that the number of known mutant loci are only 1 to 2% of the total number of mouse genes despite intensive work on induced mutagenesis with ENU (Nethyl-N-nitrosourea) on spermatogonial stem cells.

It seems that the phenotype gap in *Arabidopsis thaliana*, the most investigated higher plant, is also very wide. Probably only 1.8% of visible markers have been described if, on average, 167 genes per megabase (Mb) are expected and about 3 visible markers per Mb have been identified (Heslop-Harrison and Schwarzacher, 1993; Vizir et al., 1994). This indicates that the percentage of visible markers in *Arabidopsis* is about 20 times lower in comparison to 44.6% in *Drosophila melanogaster*, in which 83 genes are expected per Mb and an average number of identified visible markers of 37/Mb (Collins, 1995). *Arabidopsis* has a very low amount of DNA per haploid genome. The rice (*Oryza sativa*) genome is about four times larger. Other cereals such as barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*) have about 37 and 115 times more DNA than *Arabidopsis* (Table 1). This indicates the amount of work that should be done to fill in the phenotype gap in higher plants, especially the major crop species. Because of the small rice genome size, large scale mutational work should be initiated with this crop. The synteny of cereal genomes can help in the use of mutated genes in other cereals.

To narrow the phenotype gap in crop plant species it is necessary to expand mutant resources in “breadth” and “depth” similarly to animal genetics (Brown and Peters, 1996). The breadth of mutant resources can be increased by recovering mutations at new loci. The depth of mutant resources can be increased by recovering further mutations at known mutated loci. Closing the phenotype gap requires efficient mutagenesis protocols and sensitive screening methods. As current mutagenesis has a great number of mutagenic agents such as various types of radiation, chemical mutagenesis, *in vitro* conditions, insertional mutagenesis, and activation of retrotransposons, the efficient and sensitive screening method is still the most limiting factor for isolation of a particular mutation.

There are many misconceptions related to frequency of specific locus mutations. Most probably, underestimation of the frequency of mutations induced by radiation or chemical mutagens leads to a very critical assessment of their usefulness in generating desired genetic diversity in plants. For the past 25 years, it has generally been accepted that the average frequency of induced mutations is approximately on the level of  $1 \times 10^{-6}$ . This figure ignores the data related to the level of spontaneous mutations which have almost similar level for higher eucaryotes (Drake et al., 1998). Consequently, too high of mutagen doses have often been used, which induced too many mutations in the nucleus of each treated cell. The generative progeny that develop from this cell segregate for many characters that may negatively influence agronomically important characters, such as adaptability and yield potential. As a result, due to the use of high doses, many mutants were selected in mutated populations but most frequently with significant modifications in parental genetic background that made their usefulness in breeding programs highly questionable. The effectiveness of mutational strategies was also compromised by improper handling of successive mutated generations due to misunderstanding of the genetic consequences of chimeric structure of  $M_1$  plants and the adoption of “diplontic selection” concept. In reality, the frequency of mutations at numerous loci is much higher, as is indicated by the frequency of mutants in the  $M_2$  generation of some crop species (Table 2).

Mutational analysis of flavonoid biosynthesis in barley was based on the Carlsberg Collection of 772 induced flavonoid mutants (Jende-Strid, 1993). These mutants could be divided into three groups: proanthocyanidin-free - 669; deficient in anthocyanin synthesis - 75; and, with increased amount of anthocyanin - 28.

Proanthocyanidin-free mutants are important for the brewing industry to prevent haze formation in beer. A large-scale genetic analysis indicated that 568 of these mutants can be localized into 28 complementation groups of *Ant* loci. Results of

complementation tests indicate that 503 mutants which can block the biosynthesis of proanthocyanidins in the grain belong to 10 loci also described as *Ant* genes. In this example, the mutational analysis led to an increase in “breadth” of mutant resources for proanthocyanidins up to 10 loci and in “depth” by recovering numerous mutations in these loci. The mutational analysis, together with previously known data, resulted in the description of the function of particular genes in flavonoid biosynthesis (Table 3).

Mutations *Ant 13*, *Ant 17*, *Ant 18*, *Ant 21*, and *Ant 22* block the biosynthesis of both anthocyanin and proanthocyanidin, and were discovered using the same selection method. Their inheritance is monofactorial recessive. It is interesting to note the significant differences in the mutation frequency of these five loci. Mutants with mutations in *Ant 13*, *Ant 17*, and *Ant 18* loci constitute about 97% of mutants belonging to this group. The reason for the very low frequency of mutations in *Ant 21* and *Ant 22* loci is unknown.

Four mutated alleles of *Ant 18* locus were used to study the molecular basis of these mutations (Olsen et al., 1993). The structural gene for dihydroflavonol reductase coded by this locus has 3176 bp. Investigated alleles, obtained by sodium azide mutagenesis, differed mainly in transitions leading to missense mutations, and no deletion was found. Four to seven substitutions were observed in each of the investigated alleles. A total of 21 substitutions per locus were described for these four alleles. This research clearly illustrates how precise mutational analysis in depth can be.

Szarejko et al. (1998) undertook mutational analysis of 333 semi-dwarf spring barley mutants. These mutants were divided into five height classes ranging from mutants below 50% to mutants between 80 to 90% of the parent variety. Initiated genetic analysis already indicated 18 new loci for semi-dwarfness and more than 60 expected new loci. Only one of the analysed mutants was allelic to *uzu* and few were allelic to *brachytic*, two known loci for dwarfness in barley.

Recent developments of gene transfer technology have enormous promise for improvement of plant productivity; however, there is a lack of available new genes which can be transferred to current high-yielding varieties and further significantly increase yield. In other words, there are no genes that have been identified which can contribute to world crop production as much as *Rht1* and *Rht2* in wheat and *sd1* (DGWG) in rice. Borlaug (1997) referred to these genes as “master genes” and concluded that, “Biotechnology may be a new window through which to search for new ‘master genes’ for high yield potential by eliminating the confounding effects of other genes.” Therefore until new master genes are discovered, alternative solutions for crop improvement must be pursued.

For example, further increases in crop yield may involve breeding for improved root systems. Not much is known about the genetics of the root system of even major crop plants. Breeding programs for high yield and adaptability have only indirectly selected favorable root systems. Nevertheless, such an approach cannot replace a gene recombination breeding program that focuses on such characters of the root system as the dynamics of soil penetration; seminal, adventitious, and secondary root numbers; total root length; weight, number, and distribution of root hairs; and many physiological characters that directly influence plant productivity. Phosphorus uptake in barley can be almost doubled by increasing root hair density and length (Gahoonia and Nielsen, 1997). The availability of phosphorous, zinc, and other elements in poor soils as well as water and nitrogen nutrition depend also on mycorrhiza associations (Barker et al., 1998). Mycorrhizal fungi transfer assimilated carbon between tobacco plants (Müller and Dulieu, 1998). These examples indicate an urgent need for further development of selection methods and for studies of the inheritance of characters related to the root system structure, function, and their linkage with other plant characters. Mutational analysis of selected root characters in breadth and depth would be the most desired approach, especially since a high frequency of induced mutations has been observed in relation to the root characters. More than 3.3% of progenies of barley  $M_1$  plants have indicated mutation in root system characters after combined seed treatment with sodium azide ( $\text{NaN}_3$ ) and N-methyl-N-nitrosourea (MNH) at the Department of Genetics, Silesian University, Poland, according to the mutagenic treatment method described by Szarejko and Maluszynski (1980). Mutant lines selected in  $M_3$  generation indicated mutations related to root hairs, number and length of seminal roots, rootlessness, and abnormal root tip development (Table 4).

Root mutants, described in maize, were obtained after mutagenic treatment with EMS and mutator MU (Feix et al., 1997). Mutants with unusual gravitropism behavior, aberrant lateral root formation, premature root degradation, and with lack of crown and brace roots were described in mutated generations. The genetic analysis of mutants indicated that the formation of the various root types and classes is controlled by different genes.

Mutational analysis has been demonstrated as a powerful tool to dissect signalling pathways for plant defence responses (Dangl et al., 1996; Yang et al., 1997). There are also several examples of the use of mutational analysis to define the physical size, organization, and the sequence complexity of the major cluster of pathogenesis-related genes or the fine gene structure e.g. downy mildew resistance genes in lettuce (Anderson et al., 1996) and for the *Mlo* locus for powdery mildew resistance in barley (Büschges et al., 1997).

Klug and Cummings (1997) in their book “Concepts of Genetics” characterized the importance of mutations in genetic research. “Mutations are the ‘windows’ through which geneticists look at the normal function of genes, cells, and organisms.” Mutational analysis followed by functional genomics should be the system for organizing these “windows” in basic and applied research.

### **Desired Genetic Diversity on Request**

Induced mutations in rice, especially for semi-dwarfness and earliness, are most often used to demonstrate the fastest way to obtain these characters in genotypes where crosses can modify particular characters such as adaptability, aroma, taste as well as requirements of local markets. More recently, mutation techniques have also been used to generate mutants with particular requirements related to quality characters where a rapid selection method is available. Very useful mutants have been obtained for fatty acid composition in rapeseed (Rücker and Röbbelen, 1994; Kott, 1995), canola (Wong and Swanson, 1991), flax (Ntiamoah et al., 1995; Dribnenki et al., 1996), soybean (Graef et al., 1988; Fehr et al., 1992; Schnebly et al., 1995), Cuphea (Knapp and Tagliani, 1991), camelina (Vollmann et al., 1997), for grain quality in rice (Kikuchi, 1994; Iida et al., 1997; Kumamaru et al., 1997) and for amylose-free starch in potato (Leij et al., 1991).

Sunflower was selected to demonstrate the potential of mutation techniques to create desired genetic variation. Contrary to other important crops there is only one variety of sunflower released in the FAO/IAEA Mutant Varieties Database (Maluszynski et al., 1991); however, it seems that induced mutations have greatly contributed to world production of sunflower and are the major tools for future modification of this crop, based on market requests. Sunflower is the fourth most important oilseed crop. Its production is 24.9 million tons and in the United States alone more than 1.8 million tons were produced in 1995/96 (Kleingartner, 1997). Traditional varieties of sunflower have 12% of saturated fatty acids and more than 87% of unsaturated fatty acids. Most of the saturated fatty acids are palmitic (16:0) and stearic (18:0). Mono-unsaturated fatty acids are represented by oleic acid (18:1) and poly-unsaturated fatty acids by linoleic acid (18:2) in amounts of about 12 and 71%, respectively. The oil content and composition varied significantly in various environments.

To meet oilseed industry requirements and to obtain high-yielding modern varieties the following modifications of fatty acids composition and other new germplasm are essential:

- Higher oleic acid content (for food and industrial purposes)
- Higher oleic but low palmitic and stearic acids content (for food and industrial purposes to obtain higher oxidative stability)
- Higher palmitic acid content (for soap products)
- Cytoplasmic male sterility (for hybrid seed production)
- Herbicide resistance

Increasing oleic acid content has been the most important step in the modification of oil quality of sunflower. This was achieved as early as 1976 by Soldatov (1976). Seed mutagenesis with 0.5% of dimethylsulphate (DMS) was performed in 1965. After selection, the mutant variety 'Pervenets,' the first high oleic content sunflower, was officially released in the former USSR in 1977. The amount of oleic acid content was increased in this variety to more than 80% due to the mutation to partially dominant allele *O1* (Miller and Fick, 1997). Most probably, this mutated gene is the only available genetic source for high oleic acid content and has been widely used for breeding of currently grown varieties of sunflower in many countries. On the basis of this germplasm, mutation work was initiated resulting in the development of mutants that meet the oilseed industry's needs (Table 5).

Selection of sunflower mutants with reduced levels of saturated fatty acids has led to the development of new hybrids called 'NuSun.' Oils derived from the new hybrids have good oil composition to make fried foods taste better and stay longer than foods prepared with traditional sunflower hybrid oils. If widely accepted by the snack food industry, the U.S. acreage of oilseed sunflowers could double by the year 2001 (Hardin, 1998).

### **Officially Released Mutant Varieties**

Of a total 1847 accessions of the FAO/IAEA Mutant Varieties Database, crop species are represented by 1357 officially released mutant cultivars and ornamental and decorative plant species by 490 mutant varieties (Maluszynski et al., *in press*). Crop mutant cultivars were mainly developed in seed propagated plant species (1284 entries), whereas vegetatively propagated crops are represented by only 73 varieties. Among the cereals (869 mutant varieties), rice (333) ranks first, followed by barley (261), bread wheat (147), maize (49), durum wheat (25), and others (54). Most of the rice mutant varieties (67.6%) were released as 'direct' mutants, i.e. direct seed multiplication of selected mutants and their subsequent distribution to farmers. In addition, some mutants such as 'Reimei' (Japan) and 'Calrose 76' (United States) were successfully used in extensive crossbreeding programs. Semi-dwarfness (129 varieties) and earliness (117 varieties) were the most often selected characters from the treated populations. The list of improved characters also contains traits desired for increasing sustainability in rice production, i.e. tolerance to cold (13) salinity (6), and photoperiod insensitivity (3).

The vast majority (201) of the directly released rice mutant varieties was induced with physical, and only 25 with chemical mutagens. Radiation was applied in 199 cases and laser mutagenesis only in the development of two mutant varieties. Among the radiation sources, gamma rays were used in 199 cases, including 37 varieties developed by chronic

gamma irradiation, followed by 14 with X-rays, 9 with neutrons, and 3 varieties with other sources of radiation. Methyl- and ethyl-nitroso urea (12) as well as ethyl methanesulfonate (9) were most commonly used as chemical mutagens to induce mutations for breeding new varieties. According to the database, the mutant rice varieties were officially released in 26 countries. The seven top countries are: China P.R. (117), Japan (46), India (31), Guyana (26), Cote d'Ivoire (25), United States (23), and Vietnam (14). The economic impact of rice mutant varieties has been reviewed by Rutger (1992) and Maluszynski et al. (*In press*).

It is always very difficult to collect and present data related to increased farmers' income coming from the cultivation of a new variety. One of the best documented calculations deals with the Japanese pear mutant variety 'Golden Nijisseiki' released in Japan in 1991. This variety, developed with chronic radiation on a gamma field, is significantly more resistant to black spot disease than the parent 'Nijisseiki.' The farmers' cooperative group calculated an additional income of almost US\$ 50 million per year by growing this variety. This sum covers all the expenditures related to the construction of the gamma field in Ohmiya-machi, its maintenance and staff salaries for 20 years of operation (Amano, 1997). Using the same approach, two other mutant varieties of Japanese pear were recently released with even greater resistance to black spot disease (Anon., 1996; Masuda and Yoshioka, 1997).

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**Table 1. Haploid genome size of some crop species in comparison with *Arabidopsis thaliana*, *Drosophila melanogaster* and *Homo sapiens*. (After Arumuganathan and Earle, 1991; Bradley and Pruitt, 1992; Heslop-Harrison and Schwarzacher, 1993 and Dolezel et al., 1994.)**

Species	Mbp
<i>Arabidopsis thaliana</i>	150
<i>Vigna radiata</i>	470
<i>Oryza sativa</i>	415-600
<i>Musa acuminata</i>	605
<i>Gossypium spp.</i>	780
<i>Nicotiana tabacum</i>	1600
<i>Glycine max</i>	1800
<i>Pisum sativum</i>	4500
<i>Hordeum vulgare</i>	5550
<i>Triticum aestivum</i>	17330
<i>Drosophila melanogaster</i>	120
<i>Homo sapiens</i>	3000



**Table 2: The frequency of desired mutants obtained in M<sub>2</sub> generations**

Crop species	Mutagen	No. of M <sub>2</sub> plants	No. of mutants	Mutants (%)	Selected characters	Ref.
cotton	gamma-rays	11,250	40	0.355	earliness	1
cowpea	gamma-rays	10,000	2	0.020	aphid resistance	2
rapeseed	EMS <sup>a</sup>	162,000	75	0.046	sylfonylurea tolerance	3
rice	gamma-rays	50,000	112	0.224	semi-dwarfness	4
soybean	gamma-rays	44,000	135	0.307	earliness	5
tomato	EMS	5,000	2	0.040	signaling mutants	6
rapeseed	EMS	39,504	1	0.003	reduced level of PUFA <sup>b</sup>	7

<sup>a</sup> EMS - Ethyl methanesulfonate

<sup>b</sup> Polyunsaturated fatty acids

1- (Saeed Iqbal et al., 1991); 2- (Pathak, 1991); 3- (Tonnenmaker et al., 1992); 4- (Futsuhara et al., 1967); 5- (Kawai, 1967); 6- (Lightner et al., 1993); 7- (Auld et al., 1992).

**Table 3: Results of mutational analysis of proanthocyanidin biosynthesis (based on data of Jende-Strid, 1993).**

Locus	Number of mutant alleles	Function
<i>Ant 13</i>	146	Regulatory gene, coding DNA binding protein, necessary for transcription of at least three structural genes: chalcone synthase, flavanone 3-hydroxylase and dihydroflavonol reductase
<i>Ant 17</i>	178	Structural gene for one of two subunits of flavanone 3-hydroxylase (see <i>Ant 22</i> )
<i>Ant 22</i>	6	Structural gene for another subunit of flavanone 3-hydroxylase (see <i>Ant 17</i> )
<i>Ant 18</i>	134	Structural gene for dihydroflavonol reductase
<i>Ant 19</i>	1	Structural gene for leucoanthocyanidin reductase
<i>Ant 21</i>	8	Regulatory gene for flavanone 3-hydroxylase and dihydroflavonol reductase
<i>Ant 25</i>	10	Putative regulatory gene for dihydroflavonol and leucoanthocyanidin reductase
<i>Ant 26</i>	6	Putative condensing flavanol-enzyme
<i>Ant 27</i>	5	Putative regulatory gene for proanthocyanidin pathway
<i>Ant 28</i>	9	Putative regulatory gene for proanthocyanidin pathway

**Table 4: Barley seedling root mutants observed in M3 generation after mutagenic treatment with sodium azide and N-methyl-N-nitrosoure<sup>a</sup>.**

Character	Mutants (No.)
Root hairs	
hairlessness, normal root length	2
hairless, reduced root length	4
very short hairs, reduced root length	2
many dense, long root hairs, reduced root length	7
Number and length of seminal roots	
only 1 - 3 seminal roots	2
reduced root length and number of seminal roots	2
Rootless	
root length reduced to 0.4 - 2.5 cm (control 13 cm)	6
Abnormal root tip development	
“swollen” root tips	4
elongated metaxylem cells	1
secondary-like meristem of seminal roots	1
reduced zone of root elongation (hair zone is directly following the meristem)	5
irregular structure of cortex	1

**Table 5: Development of new germplasm with desired characters through mutagenesis of high oleic content sunflower.**

Objective	Character				References
	Fatty acid composition (%)				
	Parent	Mutant (or recombinant)			
Higher oleic bu low palmitic and stearic acids content	Saturated	12	Saturated	6-9	(Miller and Fick after Hardin, 1998)
			Oleic	65	
			Linoleic	26	
Higher palmitic acid content	Palmitic	3	Palmitic	30	(Fernandez-Martinez et al., 1997)
		3	Stearic	3	
	Stearic	88	Oleic	56	
	Oleic	6	Linoleic	6	
	Linoleic				
Objective	Male sterility			References	
	Type	(No. of mutant lines)			
Cytoplasmic male sterility	CMS*	22		(Jan and Rutger, 1998)	
	NMS*	7			
Objective	Herbicide resistance			References	
	Herbicide	Selected mutants (in percent of M2)			
Sunflower with resistance to herbicides	Bifenox	0.6		(Berville et al., 1992)	
	Roundup	2.3			

\*CMS- cytoplasmic male sterility; NMS - nuclear male sterility.

# **RICE GERmplasm EVALUATION AND ENHANCEMENT IN INDIA: ISSUES, STATUS, OPTIONS, AND FUTURE PLAN OF ACTION**

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## **ABSTRACT**

The Indian subcontinent has very rich diversity in rice germplasm which includes land races, wild *Oryza* species, related genera, natural hybrids between the cultigen and wild relatives, and the germplasm resources generated in the breeding programs. Through the various exploration missions, about 66,000 accessions have been collected. The large-scale spread of modern, high-yielding varieties and changes in cultural practices is leading to narrowing of the genetic base of rice agriculture. Wild species of rice are threatened with extinction as a result of environmental degradation.

The value of collected and conserved rice germplasm in terms of useful economic traits has been demonstrated in many screening tests. A network project launched in the country has evaluated more than 12,000 accessions and identified accessions possessing tolerance/resistance to pests and pathogens and to different abiotic stresses. Germplasm characterization and evaluation, complemented by biosystematic studies using the wild species and also molecular fingerprinting studies of genetic diversity, is generating the information base for more efficient use of these valuable resources.

Prebreeding or genetic enhancement has become a necessary and planned part of all rice breeding activities. However, a large number of germplasm accessions are awaiting proper evaluation and characterization. A decentralized yet integrated, harmonized, and well-organized systematic evaluation and enhancement is to be conducted in a time-bound mission-mode manner, with the participation and partnership of all the stakeholders to develop durable and stable varieties with multiple resistances/tolerances.

Breaking the yield ceiling through genetic improvement becomes the priority in rice research. Exploitation of heterosis through hybrid rice breeding provides a bright opportunity for increasing the rice yield potential in tropics. Hybrids between elite tropical indica varieties and the new plant type of tropical japonicas are being developed. The ideotype approach to plant breeding, using physiological attributes as selection criteria, is also required. Improving the productivity of rice cultivation shall involve screening of available germplasm for tolerance to biotic and abiotic stresses, and intensification of research on the genetics and physiological mechanisms of tolerance to these stresses would be important. To meet the diversified needs, consumer compatible varieties on a time scale would be desired.

The collected and evaluated germplasm shall only be used if there is a proper documentation system to consolidate and disseminate the generated information. In addition, in view of the existing and fast emerging stringent IPR regimes, it is essential that facilitated access to genetic resources for uninterrupted varietal improvement is ensured. In a benefit-based IPR regime, access may not be facilitated unless there is fair and equitable benefit sharing on account of materials used in products developed and benefit accruing thereof from their commercialization.

To fully use the diversity, the germplasm centers should have a Genetic Resources Unit (GRU) where the accessions are maintained and genetically enhanced, and provided to the breeders. Molecular characterization of released varieties and the elite genetic stocks is also to be taken up in a phased manner.

Biotechnology-led rice germplasm enhancement offers an avenue for partnership between the public and private sector with proportionate benefit sharing. Ecological risks, however, would always need to be evaluated on a case-to-case basis to understand the possible potential and likely problems, if any.

## **INTRODUCTION**

### **Production**

India already dedicates more of its precious land to rice than any other country: a whopping 42 million hectares. Planting more land to rice is definitely out of question. The solution must come from squeezing more grain from each plant through proper exploitation of germplasm diversity.

By the year 2025, the world may be required to produce about 765 million tons of rough rice as compared to 519.63 million tons it produced in 1995. More than 90% of the needed extra output has to be from Asia, where most of the world's poorest people live. Such an increase must come from higher productivity on a long-term sustainable basis.

The production environment in the future will be very different. Rice will have to be grown with less water, less labor, and on much less land. The yields must be increased considerably, that too may be under increased biotic and abiotic pressures.

Yield potential of irrigated rice in the tropics has stagnated at around 10 t ha<sup>-1</sup> since 1966 when the first semi-dwarf indica variety, IR8, was released. Rainfed rice constitutes about 45% of the total rice-growing area in Asia. Its present average yield of 2.0 t ha<sup>-1</sup> should be increased to about 4.0 t ha<sup>-1</sup> during the next 30 years. This increase is also essential to release the pressure on irrigated rice. Water- and soil-related stresses are the constraints to increase in rainfed rice production.

### Diversity

Availability of diverse germplasm is to be ensured through systematic collection and conservation of landraces and wild species that provide the genetic building blocks for plant breeding.

The populations of landraces, though predominantly self-pollinated, are genetically heterogeneous. They are also differentiated into varieties, showing a high index of gene differentiation. Traditionally, there had been a large number of such varieties in each locality before the expansion of high-yielding varieties (HYVs). Along with wild and weedy rices, these primitive varieties could be evolutionarily highly active.

The diversity of rice genetic resources is depleting. The large-scale spread of modern, high-yielding varieties and the replacement of traditional varieties, particularly in the irrigated rice ecosystem, is leading to concern that the genetic base of rice agriculture has become too narrow.

Changes in cultural practices where flood-prone systems are being replaced by irrigated ones will lead eventually to the loss of distinct deep-water rices. Wild species of rices are threatened with extinction as a result of environmental degradation.

This genetic erosion is taking place at a time when new tools of biological research enable scientists to focus as much on the diversity of genes as on the diversity of genotypes. Although there has been a considerable success in protecting and preserving the biodiversity of rice during the past decades, much remains to be accomplished.

India has abundant resources of wild rices particularly *O. nivara*, *O. rufipogon*, *O. officinalis*, and *O. granulata*. The wild species of rices can be found in many different natural habitats, from shade to full sunlight, and can be either annual or perennial in nature. Some wild species occur as weeds in and around rice fields and even hybridize naturally with the cultivated forms. This complex association between cultivated and wild forms can enhance the diversity of rice crop in traditional agricultural systems, where farmers often grow mixtures of varieties, to provide a buffer against the risk of complete loss of crop due to biotic and abiotic stresses. The complex association between cultivated and wild forms can obviously enhance the diversity of rice crop in traditional agricultural systems.

Diverse ecological situations in areas of rice cultivation have given rise to the following major ecospecific rice varieties with specificity for season, situation, and system:

- The *aus* group: Early maturing, photoinensitive types - can be grown across the seasons except in the winter.
- The *aman* group: Late types mostly photoperiod sensitive and flower during specific time regardless of when they are sown or transplanted.
- The *boro* group: Perform best as a summer crop. When sown during winter they tolerate cold temperature in the early vegetative stage better than the other groups.
- The *gora* group: Short duration, can withstand a certain degree of moisture stress during its growing period.
- The *basmati* group: Specific to regions in the northern parts of Indian subcontinent, possessing extremely valuable quality traits like elongation, aroma, flavor, etc.

According to an estimate, about 50,000 land races of rice are expected to exist in India. A total of 66,745 accessions have so far been collected from various parts of the country. If it is presumed that almost 50% of the total germplasm are duplicates, about 17,000 land races of rice still remain to be collected. Thus, a serious effort needs to be undertaken in this direction.

### Evaluation and Use of Diversity

Plant breeding relies on systematic screening, selection, and cross breeding in order to exploit the diversity of rice and combine desirable traits to meet farmer and consumer needs. This progress in rice improvement is dependent to a large extent on the diversity of materials made available to the rice program scientists.

During the past 30 years, rice improvement efforts have been directed towards incorporating disease and insect resistance, shortening growth duration, and improving grain quality. Past success in increasing yield potential has mainly been the result of an empirical selection approach, that is, selecting yield per se. Resistance/tolerance to pests, diseases, and different abiotic stresses has contributed but far less than the existing and emerging potential.

Proper documentation of basic “passport,” characterization and evaluation data about the collected and conserved germplasm is a prerequisite for its use, and also for protecting the sovereign rights. Passport data should include the national accession number, accessions’ sampling date, collector number, site of origin, ethnobotanical information on the history, and local uses of germplasm. The characterization data refer to taxonomic information that describes the stored germplasm. The evaluation data refer to agronomic properties of the accession generally based on standard descriptors.

Recently, prebreeding - genetic enhancement - has become a necessary and planned part of all plant breeding activities, and an essential part of germplasm diversification strategy. Genetic enhancement by accumulating favorable genes/alleles is needed:

- To prevent genetic uniformity and consequently genetic vulnerability.
- For raising yield levels to new heights. It is true that most breakthrough cultivars have highly diverse parentage, for example the high-yield dwarf rices. In each case, extensive prebreeding preceded development of the breakthrough high-yield cultivars. Prebreeding was used to adapt diverse kinds of germplasm to new genetic backgrounds and new geographic locales.
- To bring in traits not found in local cultivars.
- To enhance the chances of success in space and time in contemplated crop improvement programs.

The advent of molecular genetics and cell biology has generated the possibility of a new kind of biotechnology-assisted genetic enhancement. The hitherto unavailable genes for insect resistance may be transferred from alien species into elite genotypes (e.g. *Bt* genes from bacteria to maize); or, cellular fusion may be used to construct new systems of cytoplasmic male sterility (e.g. in Brassica species); or, restriction fragment length polymorphisms (RFLPs) may be used to build up information on RFLP linkages with agronomically useful traits (e.g. as in wheat, to allow faster introgression of alien germplasm). Such novel genetic potentials have raised the possibility of entirely new product lines - new kinds of plant cultivars, never before available; and has attracted the attention of entrepreneurially inclined scientists and business people.

While *ex situ* conservation in a genebank is the most secure and cost-effective strategy for the long-term preservation of the rice gene pool, and germplasm conserved in this way is immediately available for researchers and breeders to use for rice improvement, other conservation strategies for rice landraces are to be developed.

## STATUS

### Diversity

The diversity of rice crop evolved over thousands of years, as the peasants and farmers - mostly women - selected different types to suit local cultivation practices and needs. This process of selection has led to a multiplicity of rice varieties adapted to a wide range of agro-ecological conditions, and with resistances to insect pests and diseases.

Weedy rices are found in directly seeded rice fields in certain regions, they might have been derived from hybrids between wild and cultivated rices. The weedy populations contain gene diversity and seem to serve as gene reservoir for cultivars.

India has naturally-occurring wild rices, particularly *O. nivara*, *O. rufipogon*, *O. officinalis*, and *O. granulata*. The habitats of *O. nivara* are ditches, water holes, and edges of ponds, whereas *O. rufipogon* is usually found in deepwater swamps.

The full spectrum of rice germplasm thus includes:

- Wild *Oryza* species and related genera
- Natural hybrids between the cultigen and wild relatives and primitive cultivars of the cultigen in areas of rice diversity.
- Germplasm generated in the breeding programs including pureline or inbred selections of farmers varieties, F1 hybrids and elite varieties of hybrid origin, breeding materials, mutants, polyploids, aneuploids, intergeneric and interspecific hybrids, composites, etc.
- Commercial types, obsolete varieties, minor varieties, and special purpose types in the centers of cultivation.

### Germplasm Collections

Exploration and collection of indigenous rice cultivar was initiated around the turn of the century. The establishment of Agriculture Research Station at Dacca in 1911 and the Paddy Breeding Station at Coimbatore in 1912 and Central Rice

Research Institute (CRRI) at Cuttack in 1946 strengthened these activities. This led to recommendation of 394 varieties for general cultivation, as pure line selections, from the collected germplasm.

Systematic exploration programs were initiated during 1955 to 1960 by the Jeypore Botanical Survey, which led to collection of 1,745 cultivars from South Orissa and adjoining areas of Madhya Pradesh. About 900 cultivars from Manipur were collected during 1965 to 1967. During 1965 to 1972, 6630 accessions were collected by the Indian Agricultural Research Institute (IARI), which came to be known as the Assam Rice Collection. The cultivars grown in the Madhya Pradesh region were collected and the 19,116 accessions collected formed the Raipur Collection. A special drive for upland paddy varieties under cultivation in Andhra Pradesh, Karnataka, Maharashtra, Madhya Pradesh, Uttar Pradesh, Orissa, and West Bengal resulted in collection of 1,938 cultivars. National Bureau of Plant Genetic Resources (NBPGR) augmented the collections during 1983 to 1989 by 4,862 accessions. In addition, joint explorations by NBPGR in collaboration with State Agricultural Universities (SAUs) during 1978 to 1980 and CRRI during 1985 resulted in collection of about 7000 and 447 accessions (from Sikkim, South Bihar, and parts of Orissa) respectively. VPKAS Almora explored the germplasm in hilly region of Uttar Pradesh and 1,247 primitive cultivars were collected.

The collections of wild rice was initiated at CRRI during 1948 to 1955. Subsequently, collections were made at IARI from western, northern, central, and eastern India. Later, variability in *Portersia coarctata* was collected from the coastal regions. Many joint explorations with other countries have also been undertaken. Japanese exploration teams led by H. Kihara in the early 1960s and T. Watabe in the late 1960s and early 1970s made systematic collections in Western Uttar Pradesh, Bihar, Andhra Pradesh, and parts of Maharashtra. H.I. Oka travelled extensively on collection trips in different parts of India. French team from IRAT and ORSTOM collaborated with ICAR for collection, particularly of *O. nivara*, *O. rufipogon*, and *O. officinalis* from Goa, Karnataka, Maharashtra, and Gujarat in 1986. During 1987 to 1989, ICAR and IIRI scientists undertook intensive collection for wild rice in South India and West Bengal.

Various collection missions and the collections made are mentioned in Table 1. Source-wise material deposited as base collection is mentioned in Table 2.

### Germplasm Evaluation and Use

The diversity of rice has been well used in efforts to solve today's food problems. Rice landraces were collected over several decades to become "parents" of the high-yielding, pest-resistant, and well-adapted varieties that resulted in unprecedented increases in rice yields. The cost of rice to millions of consumers is now approximately half to what it was in 1960 because of these gains in productivity.

The value of collected and conserved rice germplasm has been demonstrated in many screening tests for useful economic traits. Thousands of accessions have been screened for resistance to eight insect pests and five diseases of rice as well as tolerance to different abiotic stresses. Resistance to some pests and diseases such as brown planthopper and rice blast is quite common in the cultivated rices. Germplasm characterization and evaluation, complemented by biosystematic studies using the wild species and molecular studies of genetic diversity, is generating the information base for more efficient use of these valuable resources.

The earliest intensive pureline selections from locally collected germplasm resulted in release of about 400 improved cultivars, yielding 10 to 20% more than the traditional cultivars and adapted to various agroclimatic regions. The japonica - indica hybridization scheme launched after 1950 by the ICAR to introduce non-lodging and fertilizer responsiveness in indigenous tall indica, released variety ADT 27. In the 1960s, the hybridization programs extensively used the introduced semi-dwarf genotypes of Taiwan possessing Dee-gee-woo-gen gene, and a number of semi-dwarf, high-yielding varieties were developed. The intensive cultivation of these semi-dwarf hybrids resulted in disease and pest problems and consequently research was reoriented toward collection and evaluation of the indigenous germplasm, and many resistance donors are identified mainly at CRRI and DRR (Directorate of Rice Research).

In 1989, The ICAR, with subsequent support also from the United Nations Development Programm, launched a national hybrid rice project that resulted in 12 strategically located hybrid research centres. Through this effort, India has released 14 tropical rice hybrids through public institutions and private seed companies. In 1997, Indian farmers planted about 100 thousand hectares with these hybrids.

At DRR, Hyderabad a large number of germplasm accessions have been evaluated for agronomic characters, including against major insect pests and diseases; and several promising accessions have been identified and used in the all India coordinated rice improvement program. All major rice research centers including CRRI, IARI, APAU, TNAU, IGKV, KAU, AAU, NEH Research Complex and others were involved in this endeavor. In addition, a network project was approved by the ICAR for the period 1993 to 1998 at DRR to evaluate available germplasm against major insects and diseases. The network had two lead Centers i.e. NBPGR and IGKV, and 13 hot spot locations, namely Almora (UP), Aduthurai (TN), Kapurthala (PB), Sambalpur (OR), Raipur & Jagadapur (MP), Maruteru & Srikakulam (AP), Mandya & Ponnampet (KAR), Moncompu (Kerala), and Pondicherry (UT). Up to 1997, more than 15,000 accessions have been screened at various hot spot locations and a catalog for 12,000 accessions with 21 characters has been developed.

Evaluation efforts have resulted in identification of useful traits viz. resistance or tolerance to tropical strains of bacterial blight, tungro virus, grassy stunt virus, ragged stunt virus, rice green leafhopper, brown planthopper, stem borers, and gall midge; and tolerance for salinity, alkalinity, flood, and submergence.

The collection-wise list of traits for which promising accessions/donors have been identified are given in Table 3.

### **Genetic Enhancement**

So far, genetic enhancement has been done by plant breeders and geneticists in the public sector, who either know that they must prebreed in order to make further progress in variety development of their crop or they like the challenge of finding, incorporating, and adapting useful germplasm from unlikely places. This has resulted in incorporation of exotic germplasm into adapted, elite stocks, and new cultivars often do contain significant segments of exotic germplasm, giving new kinds of disease and insect resistance, new levels or kinds of stress tolerance, or greater yield capability.

The International Rice Research Institute (IRRI) which has provided cultivars and advanced breeding materials for use in developing countries has made an important contribution. Today, as national breeding programs are maturing, the national germplasm resource centers will have to reorient their missions.

### **Biotechnological Approach**

Commercial applications of biotechnology in prebreeding activities are gaining grounds. Its development generally has been along one of the following two lines:

- Some companies have developed fully integrated research and development programs, designed to use a combination of molecular biology and standard plant breeding techniques for prebreeding and final cultivar production. Their intention is to turn out and market finished cultivars with novel traits or with greatly improved existing traits.
- Other companies (or sometimes the same companies) have opted to use molecular genetics (for example, genetic transformation) or cell biology (for example, selection in tissue culture) to modify - to genetically enhance - existing elite germplasm stocks which then can be marketed as source materials for development of new cultivars containing the novel, engineered traits. The “engineered genetic enhancement” may be done on contract, or it may be done with expectation that the new enhanced genotypes will be marketed (via sale or licensing) to end-users such as seed companies or integrated food processors. In either case, genetic enhancement is done as a commercial end in itself; the end-users, the purchasers of the enhanced materials, will do the final step of cultivar development.

These biotechnology-using genetic enhancers generally do not depend on public funds; they intend to be supported by profits from sale or licensing of their enhanced germplasm by the seed companies that use their products.

## **OPTIONS AND PLAN OF ACTION**

### **Augmenting the Germplasm Collections**

Based on past experience, and present scenario of genetic erosion being fast in recent years, the collection and conservation of rice germplasm deserves urgent attention. Priority collection trips are to be made in the identified areas not sufficiently covered so far and a repeat collection shall be made in other areas that showed extensive diversity in the past. More emphasis shall be given in future on trait specific explorations.

There has been a deficiency in sampling the wild rices during the earlier missions when the focus was on the landraces and traditional cultivars. In addition, different approaches and strategies are required to be adopted for wild rices, which exhibit rich genetic diversity. They have been shown to be rich sources of useful genes for resistance to pests and diseases. The improved wide hybridization technique, the desire to explore new sources of genetic variability for improvement of the major cultivars, and the threat of extinction of the wild populations are the other indicators, which suggest that the demand for wild germplasm will increase markedly. Rapid development in the techniques for use of germplasm equally requires the prioritization of regions for collections of specific diversity.

### **Evaluation and Use of Germplasm**

A large number of germplasm is awaiting proper evaluation and characterization. A decentralized yet integrated, harmonized and well-organized systematic evaluation of this genetic wealth in the shortest possible time will help to develop durable and stable multiple resistance varieties. The activity is to be conducted in a time bound mission mode manner, with the participation and partnership of all the stakeholders including ICAR and other public sector research institutes, SAUs and general universities, NGOs and private research organizations/foundations. The public sector institutions should in-

volve the NGOs/research foundations/farmers for on-farm participatory plant breeding projects, more so in the marginal productivity areas where the traditional landraces are still being preferred by farmers over the HYVs.

Since 75% of all rice is produced on irrigated land, breaking the yield ceiling of irrigated rice through genetic improvement becomes the top priority in rice research. The yield of current semi-dwarf varieties is limited largely by dry matter production. It can be improved by modifying the present high-yielding plant type and thereby by increasing the harvest index. Further the approach could be to modify the basic physiological processes governing biomass production, such as photosynthesis. Reducing photoinhibition will increase the operational photosynthetic rate to its intrinsic maximum rate and delaying leaf senescence can extend photosynthetic duration. High dry matter accumulation coupled with selection of large panicles will lead to an increased sink size, which is defined as spikelet number per unit ground area. Grain filling has to be improved to convert the increased sink into additional grain yield. Extension of grain-filling duration and further optimization of panicle morphology are the first steps to increase filled grain percentage. Translocation efficiency of stored assimilates, transporting rate, and the ability of spikelets to accept carbohydrates should also be improved. Lodging is another yield-limiting factor, especially at high yield levels. Selection for thick and sturdy stems and lowering panicle height could partially improve lodging resistance. Studies on chemical composition and physical structure governing stem strength are needed.

The ideotype approach to plant breeding, where, a plant type, which is theoretically efficient, based on knowledge of physiology and morphology, is defined first and breeders then select directly for the ideotype, rather than select only for yield is required. It is expected that during the next decades genetic improvement of yield potential will be accelerated using physiological attributes as selection criteria.

Breeding for new plant type using tropical japonica germplasm has resulted in a phytotype with increased sink size due to large panicles, less unproductive tillers, sturdy stems, and erect leaves. The yield potential of this new plant type is limited by poor grain filling.

Exploitation of hybrid vigor or heterosis through hybrid rice breeding provides a bright opportunity for increasing the rice yield potential in the tropics. Intervarietal hybrids between indica rices have demonstrated a yield potential of 11.2 t ha<sup>-1</sup> under tropical conditions. Intersubspecific hybrid between indica and japonica showed higher heterosis for yield than indica/indica hybrids. Hybrids between elite tropical indica varieties and the new plant type tropical japonicas are being developed.

There is an urgent need to intensify research on the genetic and physiological mechanisms of tolerance for abiotic stresses. Tolerance for the abiotic stresses is available in the germplasm. Various combinations of these tolerance traits that can match the many different subecosystems are needed to increase and stabilize yields. The success so far in developing improved varieties tolerant to abiotic stresses has been limited. The main reasons are that understanding of the physiological mechanisms and the inheritance of these traits is inadequate, and rapid and reliable techniques for screening genotypes, specially for multiple stresses, are lacking. Some progress has been made with respect to tolerance for flooding and salinity. But for drought and other soil stresses, progress is very little. This research is also essential to explore the new genetic engineering opportunities, such as marker-aided selection techniques that seem to offer a promising solution to breeders.

### **Strengthening the Database**

The information regarding the available and evaluated germplasm is at present scattered as it is generated at various sites. There is an urgent need to consolidate it through a national database system. The system should establish links between all those involved in the conservation, management, development, and use of plant genetic resources. This network shall serve to set priorities for action, develop policies, and provide means whereby crop-specific and regional views can be shared and exchange and use of plant genetic resources promoted.

NBPGR provides IC numbers to the indigenously collected germplasm. Breeders and others concerned should not only ensure that the material in their possession is provided national accessioning number but that these numbers are retained whenever the accession is referred to. Information on the use of germplasm needs to be documented by all agencies in order to safeguard the national interests in light of WTO/CBD requirements for access and benefit sharing. In view of the existing and fast emerging stringent IPR regimes, it is essential that facilitated access to genetic resources for uninterrupted varietal improvement is ensured. In a benefit-based IPR regime, access may not be facilitated unless there is a fair and equitable benefit sharing on account of materials used in products developed and benefit accruing thereof from their commercialization. Even internal exchange of PGR may require MTAs/LoAs.

### **Germplasm Enhancement**

Still only a fraction of the existing diversity has been used. In order to encourage better use of the germplasm, there is a need to develop core collections. Proper human resources will have to be generated for this purpose for creating a group of scientists or professionals who are true "genetic enhancers," or "prebreeders." The germplasm centers should have a GRU, which should be active sites for germplasm repositories and these should choose potentially useful materials in their



collections (of landraces and/or wild related species) and then genetically enhance them for use by others for cultivar development. The notion that neither fame nor fortune comes to those who do only prebreeding will have to be reversed. Instruments of recognizing their accomplishments such as through registration of genetic stocks developed by them should also be made fully functional. Molecular characterization of the released varieties and the elite genetic stocks should also be taken up in a phased manner. Needless to emphasize that generous funding for these activities will also have to be provided for.

Private breeders usually depend on the public sector to incorporate needed exotic genes, to remold exotic strains into adapted breeding materials. Genetically enhanced stocks, by public breeders for their own use in cultivar production, have been freely available to private breeders as well. In the changing global scenario under the IPR regimes, modalities for compensation to these public institutions and the breeders will also have to be worked out.

The public sector institutes are the custodians of the major germplasm holdings, the raw materials for any biotechnology-based enhancement. A partnership of the public and private sector can be developed, so envisaging proportionate benefit sharing. However, up to this time, biotechnology-based genetic enhancement business has been supported mainly by venture capital funds, not by sale of genetically enhanced products, and such arrangements necessarily must be made with no knowledge or experience of how much added value really will accrue from the genetically-enhanced materials, or when such value might be ready for sale in products, or whether farmers would want it, or be willing - and able - to pay for it.

Even if genetic enhancement does become a commercially successful enterprise, publicly funded efforts will continue to be needed for those basic, preliminary kinds of genetic enhancement (early prebreeding) that require long-term effort with uncertain or even unpredictable results. Such long-term, chancy work will not attract private enterprise. Thus, for well-rounded genetic enhancement programs public research in genetic enhancement will always be needed.

### **Biotechnological Approach**

Biotechnology - the sum of the technologies deriving from molecular and cell biology - will have use in plant breeding even beyond the prebreeding stages. Genetic transformation - nonsexual insertion of alien genes and/or gene regulating systems - is envisaged as eventually being applied, quickly and easily, to finished cultivars, to endow them with a series of useful new traits. Long periods of trial and error are needed to bring transformed lines into desired levels of expression. In addition, the associated ecological risks, with the introduction of transgenics, would always need to be evaluated. However, for some years to come, genetic transformation perhaps will be used only as a prebreeding technique, one in which alien genes are put into breeding pools of elite germplasm, and standard plant breeding methods are used to extract good new varieties containing the alien genes. Cell culture techniques particularly protoplast fusion techniques - will have use for making new combinations of nuclear and cytoplasmic genes.

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**Table 1. Rice germplasm collections in India.**

S. No. Agency/ Area	Year	No. of collections
1. Base collection from Coimbatore (AC)	1911-47	2,000
2. Jeypore Botanical Survey (JBS)	1955-59	1,745
3. Manipur Collection (MNP)	1965-70	904
4. West Bengal Collection Chinsurah, Bankura and Kalimpong	1960-71	5,550
5. Assam Rice Collection (ARC)	1968-71	6,630
6. Dr. Richharia's Collection from M.P.	1971-81	19,000
7. Collection from various states (Upland)	1976-77	1,431
8. National Collaborative Program (NCS)	1978-80	6,439
9. U. P. Hill collection by Pantnagar Univ. and VPKAS, Almora	1978-90	2,580
10. Lowland and saline types from Orissa	1980-82	300
11. Collection by NBPGR from different parts of India	1976-84	8,353
12. Collection by P. A. U., Kapurthala	1982-84	1,178
13. Collection by H. A. U., Kaul	1982-86	960
14. Collection by NBPGR/ CRRRI from Goa, Gujarat, Karnataka, Sikkim, Mizoram, Arunachal Pradesh and Orissa	1984-88	3,092
15. Collection from Rajasthan and Maharashtra	1980-90	3,489
16. Collection by NBPGR from Orissa, W.B.	1986-95	3,122
17. Collection by CRRRI from Orissa	1990-95	717
18. Wild rice Collection by NBPGR/ IRRI/ IGKVV/ CRRRI	1984-97	840
Total		68,330

**Table 2 . Number of Germplasm Accessions deposited in National Gene Bank.**

CENTER	NO.OF ACCESSIONS
NBPGR, TRICHUR	1,086
NBPGR, CUTTACK	1,299
NBPGR, SHILLONG	1,270
NBPGR, BHOWALI	270
NBPGR, NEW DELHI	15
IARI NEW DELHI	20
ICAR RES.COMP.,TRIPURA	60
DRR, HYDERABAD	3,900
CRRRI CUTTACK	21,874
IGKVV, RAIPU	8,712
MSSRF, CHENNAI	43
IRRI PHILIPPINES	3,455
Total	42,004

**Table 3. Traits for which germplasm has been evaluated and donors identified among the various collections in the country.**

COLLECTION	TRAITS	
CRRRI (old collection)	Abiotic stresses	Upland with adequate moisture, Upland with moisture stress, Saline and alkaline soils, low-lying areas with waterlogging flood and deep water conditions
	Biotic stresses	Blast, Helminthosporiosis, Bacterial leaf blight, Stem rot, Stem borer, Gall midge
CRRRI (JBS collection)	Agronomic / yield	High no. of grains per panicle, high 1000-grain weight, Early and good tillering, Scented kernel
	Biotic stresses	Blast, Bacterial blight, Gall midge, RTV
ARC	Agronomic / yield	Dwarf with Indica type grain, Dwarf with Japonica type grain, Javanica plant habit, Glaberrima type plant habit
	Abiotic stresses	Tolerant to flood
	Biotic stresses	Blast, Bacterial blight, Stem rot, Stem borer, Gall midge, RTV, WBPH, brown plant hopper, Multiple resistance to-GM,SB, BLB, BLS, BL, RTV
DRR / ARC	Biotic stresses	Blast, Bacterial leaf blight, Stem borer, Gall midge, RTV, Green leaf hopper
DRR	Biotic stresses	Blast, Bacterial leaf blight, Sheath blight, RTV, Green leaf hopper, Stem borer, WBPH, Brown plant hopper, Gall midge, Leaf folder, Yellow stem borer
ICAR Research Complex for NEH Region	Agronomic / yield	Rainfed upland, lowland, earliness, Non-lodging tall habit, fine grain, glutinous rice, scented rice, Suitable for high altitudes (1300m), Suitable for medium altitudes (800-1300m), Suitable for low altitudes (<800m)
	Abiotic stresses	Drought tolerance, tolerance to cold at flowering, tolerance to iron toxicity,
Raipur	Biotic stresses	Blast
	Abiotic stresses	Drought
	Biotic stresses	Bacterial blight, Panicle midge, Stem borer, Gall midge, WBPH, Brown plant hopper, Brown spot
Assam	Agronomic / yield	High protein content, High aromatic oil content
	Abiotic stresses	Drought tolerance, flood, submergence, low light
	Biotic stresses	Blast, Bacterial leaf blight, Sheath blight, Root knot nematode, Brown plant hopper,

## IMPROVEMENT OF RICE PRODUCTIVITY VIA WIDE CROSSES IN KOREA

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### ABSTRACT

A systematic rice-breeding program through hybridization was started in 1915 and has released 173 cultivars in Korea. Hybridization within the japonica population had not led to a breakthrough for yield potential by the 1960s. The successful development of semi-dwarf rice cultivar 'Tongil' through a wide cross between indica and japonica cultivars dramatically increased rice yield by 30% during the 1970s. Major genetic advances were repatterning plant architecture to one more suitable for high photosynthesis, high nitrogen response, high harvest index, and lodging resistance. Indica cultivars contributed to improved disease and insect resistance in the indica/japonica wide hybridization. Common incidence of hybrid sterility and the accompanying undesirable traits were overcome through the cross system of indica//japonica /indica instead of the previously employed system of japonica//japonica /indica or japonica//indica/japonica. The indica/japonica breeding program has contributed to improving japonica cultivars for short-stature and pest resistance as well as yield potential. Yield potential of modern japonica cultivars has remarkably increased up to 5.5 tons/ha in milled rice. However, the additional breakthrough of yield potential may not be easy in the future. Wide hybridization of different ecotypes in the cultivated species showed a superiority in total dry matter, grain yield, and potential yield depending upon the cross combinations of the different ecotypes used as parents. The interspecific wide cross will also offer the opportunities to increase biomass and grain yield as well as resistance and/or tolerance to biotic and abiotic stresses. The embryo rescue techniques are available to overcome the incidence of interspecific reproductive abnormalities.

### INTRODUCTION

Rice production has nearly doubled during the last 50 years, while hectareage has declined due to industrialization and urbanization in Korea. The current high yield of 5.18 ton of milled rice in 1997, resulted from a combination of the genetic improvement of varieties and intensive cultural practices.

A series of rice cultivars from crosses of indica/japonica, led to the Tongil cultivars, which dramatically raised yield potential and helped achieve the green revolution in Korea during the 1970s. Genetic advances in rice productivity have resulted from enhanced resistance to pests and the modification of plant type including semi-dwarfism and, canopy architecture for higher photosynthetic ability (Park et al., 1990).

Another breakthrough in yield potential may not be easy, but could be achieved by further modifying the existing high yield plant type to increase biomass, harvest index, early vigour and reduced uneffective tillers. The combination of improved plant architecture and heterosis has been suggested as another means to the jump yield potential of rice in the future (Khush, 1995).

The breeding needs for further improvement are the exploitation of germplasm, crosses between different ecotypes within cultivated species, and wide hybridization to incorporate desirable genes from wild relatives. Unlike other cereals, the introduction of desirable characters from wild relatives into rice has not been common due to the availability of genetic diversity within cultivated species and interspecific hybrid sterility. Recent advances such as embryo/ovule rescue techniques following backcrosses have allowed the use of wild relatives in rice improvement (Jena and Khush, 1984; 1986).

In this paper, the authors describe the successful improvement of yield potential remote crosses of indica/japonica cultivars in Korea, and discuss some research results aimed at the further improvement of rice yield through intraspecific and interspecific hybridization.

### **Rice Environments and Breeding Progress**

Rice is grown on about 1.1 million hectares, 54% of the total areable lands, in Korea and total production exceeds 5 million M/T per annum. As a staple food grain, rice provides 40% of calories consumed by 45 million people and accounts 49.2% of the farming incomes in rural areas.

Most of the rice is grown in the lowlands and irrigated mainly with surface water. Most rice is transplanted using a machine transplanter. Direct-seeding as a means to cut production costs and overcome the shortage of farm labor started in 1992 and had expanded to 10% of total rice lands by 1997.

Rice is grown from mid-April to mid-October and often suffers from various biotic and abiotic stresses. During the rice season, mean monthly temperatures gradually increase from around 13°C in April to above 25°C in the warmest month of August and conversely decreases by below 13°C in October. This environment often causes cold injury, both at the beginning and the end of the growing season, especially in the hilly and northern plain regions. The most severe cold damage occurred in 1980 and 1993, with estimated yield losses of 34 and 9%, respectively.

Even though it is very difficult to access the actual damage, the average annual yield loss over the past 20 years has been estimated at 3.8% to diseases and 2.3% to insects. Major diseases are blast, sheath blight, bacterial blight, and stripe virus. Warm temperature and high humidity during the middle of rice cropping in July and August can cause a widespread blast epidemic. Major insects are brown planthopper, whitebacked hopper, stem borer, and rice leaf folder. Recently, the rice water weevil has emerged as one of the important insect pests. In general, the genetical changes in rice cultivars and the changing cultural practices including high nitrogen, wider range of planting time, and methods to change the incidence of pests.

Geo-ecological regions of rice in Korea are sectioned into five ecosystems with respect to a combination of climatic ecosystem and maturity of rice cultivars as follows :

- 1) South-western and South coastals with the late maturing cultivars (=130 days to heading)
- 2) South inner-plains with medium-late maturing cultivars (116~125 days)
- 3) Central plains and South-eastern coastals with medium maturing cultivars (106~115 days)
- 4) Northern plains and hilly regions with early maturing cultivars (96~105 days)
- 5) Alpine and high elevation regions with very early maturing cultivars (=95 days)

In addition to the above five ecosystems, short-duration cultivars are currently used across ecosystems mainly in rice-based cash crop double cropping systems where rice is transplanted in late June and early July. Thus, the essential characters of rice cultivar in these areas are short-growth duration of less than 95 days with cold tolerance.

Breeding objectives vary with the ecology and the prevailing production constraints. Various social aspects significantly influence on the diversification and modification of the breeding objectives.

The basic objectives of rice breeding programs in Korea are superior grain quality, high yield potential, pest resistances, and tolerance to cold, drought and salinity stresses.

The comprehensive breeding program on rice varietal improvement in Korea was initiated in 1906 with performance tests of native collections and followed by adaptability tests of foreign introductions. Systematic rice breeding through hybridization started in 1915, and the first rice cultivar via hybridization was released in 1933. Since then, a total of 173 cultivars have been released.

Among the various ecotypes of the Asian rice cultigen, japonica rices, which are mainly grown in temperate areas, have been well adapted in Korea. By the 1960s, only temperate japonica rices were cultivated and used as major genetic resources for varietal improvement in Korea. Those traditional japonica cultivars that originated from Korea and Japan lodged easily, were tall, low in photosynthetic ability due to poor canopy architecture, susceptible to pests, and lower yield potential. Thus, the breeding program emphasized improved plant architecture, nitrogen response, lodging and pest resistances, and stress tolerances rather than yield potential itself.

The successful development of a rice cultivar Tongil through indica/japonica hybridization was a breakthrough in yield potential. Since then, rice breeders in Korea have extensively used indica germplasm to incorporate desirable characters like semi-dwarf, pest resistance and better plant architecture through the intervarietal recombination of indica and japonica. These successes proved the importance of genetic diversity in the rice cultigens for rice varietal improvement. It was a milestone and opened a new frontier of rice breeding in Korea.

During the 1980s consumers preferences for higher grain quality with japonica-like eating quality have led rice breeding plan to return back to improvement of traditional japonicas.

### **Impact of Indica/japonica Variety**

Narrowing of the genetic basis became obvious when the limited japonica cultivars were grown and used as genetic

resources during the 1960s in Korea. Thus, a large breakthrough in yield potential was not successful in the 1960s even though there was gradual improvement.

A remote cross between indica and japonica germplasm was implemented from the middle of the 1960s to breakthrough yield potentials. With the close cooperation of IRRI, a semi-dwarf Tongil cultivar was released through a cross between indica and japonica rice in 1972. The photoperiod insensitivity of the semi-dwarfs has made it possible to grow the new cultivar in a short temperate growing condition of Korea. The high yield potential of the Tongil cultivar was proved by yield trials of 2,750 ha on 550 pilots farms.

The semi-dwarfing, high-yielding Tongil cultivar, which was resistant to lodging and blast and stripe virus, rapidly spread and was grown in 0.3 million hectares in 1974 (Choi et al., 1974). However, several drawbacks of the Tongil cultivar such as cold susceptibility and unfavorable grain quality which were derived from the indica germplasm became the limitation of further spread to other growing areas. The first indica/japonica derived cultivar, Tongil was replaced by later "Tongil type" varieties with improved grain quality, enhanced levels of pest resistance, and had earlier maturity. The continued efforts of rice breeders have resulted in the release of 40 improved cultivars from indica/japonica crosses, and has helped maintain self-sufficiency of rice in Korea.

Success of Tongil and many later improved Tongil type cultivars proved that crosses between indica and japonica rices was worth while in rice improvement and implied the importance of diverse genetic variations in the rice cultigens for rice varietal improvement, especially in the incorporation of specified traits into japonica rice.

### **Breeding Procedure**

Tongil was released in just seven years through rapid generation advances of Korea-IRRI cooperative shuttle breeding, and was a breakthrough when compared to the 15 years of breeding required to develop a variety was conventional hybridization procedures used at that time in Korea.

It was developed through a three-way cross. A single cross (IR568) was made between Yukara, an early maturing and cold tolerant japonica, and Taichung Native 1, a semi-dwarf indica were adapted to the tropical region. The F1 was subsequently top-crossed to IR8, an improved indica cultivar with semi-dwarf short stature and high yield potential. A three way cross was obviously required since the single cross between japonica and indica generally showed high hybrid sterility. After growing both F1 and F2 generation at IRRI, the F3 lines were grown in Korea during the summer of 1967. Selection was continued at IRRI during the winter season and in Korea during the summer season. In 1970, three promising lines, Suweon 213, and Suweon 213-1, and Suweon 214 were selected from the pedigree of IR667-98-1-2-2 and were evaluated in replicated yield trial nurseries at three crop experiment stations in Korea. In 1971, the most outstanding line Suweon 213-1, were finally selected from the regional adaptability test at 68 sites over Korea and nominated as a new cultivar, Tongil, which means the unification in Korean (Choi et al., 1974).

The main factor responsible for this success was the crossing scheme between the intervarietal group. Common incidence of hybrid sterility and accompanying undesirable traits in crosses were overcome through the cross system of indica/japonica/indica instead of the previously employed scheme of japonica/japonica/indica or japonica/indica/japonica. In the past, indica was crossed once to japonica as a donor of disease or insect resistance and subsequently backcrossed several times to the japonica as a recurrent parent to produce a japonica-like cultivar, in which other desirable characters were not be incorporated (Chung and Heu, 1991). This new cross scheme between different rice ecotypes seemed to give more opportunities for diversifying genetic variabilities as well as overcoming the cross sterility.

### **Improvement of Yield Potential**

Annual rice production was far behind the amount needed to meet self-sufficiency in the 1960s. It is certain that the genetic improvement in rice varieties has played a leading role in increasing rice production with no significant changes in the total rice area.

Rice yield potential was improved incrementally through conventional pedigree breeding from crosses between closely related japonica elite germplasm. The national average yield productivity of rice cultivars increased from 2.98 in the 1930s to 3.98ton/ha in the early 1970s with the crosses between closely related japonica elite germplasm and was equivalent to 0.8% point increase per annum. Production increased dramatically to 5.13 ton/ha when the "tongil" cultivar was released in 1972. It was 30% higher than the best commercial japonica cultivar at the time and 72% higher than the native collections and foreign introductions in the 1930s.

The continued effort of rice breeders has resulted in the release of 40 cultivars through crosses between indica and japonica varieties. The milled rice yield of the subsequent Tongil type cultivars steadily increased from 5.13 t/ha for Tongil in 1972, to 5.76 for Millyang 23 in 1976 and up to 6.05 for Yongmoonbyeon in 1985 (Park et al., 1990). The acreage of these Tongil type high-yielding cultivars increased sharply from 22.9% in 1975 to 76.2% of the total rice acreage in 1978, and helped achieve the green revolution in Korea (Table 1).

The higher yield potential of the indica/japonica rice cultivars was mainly based on the genetic improvement of canopy architecture for higher photosynthetic ability with high leaf area index (LAI), improved light transmission rate (LTR) with leaf erectness and proper leaf angle, and high translocation of photosynthates with larger vascular bundles (Kwon, 1988; Lee and Shu, 1993; Park, 1988). Other genetic advantages of indica/japonica hybridization were semi-dwarf short stature with lodging resistance, high nitrogen response with high tillering and larger panicles, and resistances to diseases and insect pests.

Other crosses between Tongil type and japonica varieties have greatly contributed to the improved plant height, short stature, pest resistance, and yield potential of japonica cultivars. The genetic improvement in yield potential of modern japonica cultivars has steadily increased over the past 15 years. The yield of the modern japonica cultivars has reached 5.5 ton/ha, which is 79% higher than that of 1930s and 34% higher than that of the 1970s.

“Dasanbyeon” and “Namcheonbyeon,” which were recently released as improved Tongil type cultivars, yield 6.77 and 6.36 ton/ha, respectively. In 1997, these new varieties were grown on 8.2 thousand hectares and yielded 7.2 and 6.7 ton/ha respectively.

### **Possibility of Rice Improvement through Intraspecific Hybridization**

Indica/japonica hybridization have permitted a quantum jump in yield potential of rice from repatterning of plant architecture during the 1970s in Korea. The yield potential of japonica rices have also increased during the last 15 years, although a large gap still exists between Tongil type and improved japonica cultivars. For another jump in yield potential, breeders must explore the possibility of further modifying the present high-yielding plant architecture (Khush, 1995).

Current challenges of rice breeders are to evaluate and use germplasm resources in unadapted materials including indica, tropical japonica (Javanica), and related wild species as well as adapted elite breeding materials. A main part of the genetic improvement of a crop resides in the creation of new genic combinations from the available germplasm.

Indica rices, which are not well adapted in temperate areas, are generally more diverse in genetic variability than japonica rices which are well adapted in temperate areas. The japonica rices, however, are distributed over a wide range of latitudes, and divisible into tropical and temperate subgroups with different morphological and physiological traits (Sato, 1987; Oka, 1988). The cultivated rice gene pools still contain adequate genetic variability to make significant improvement in productive traits probably without restoring to the use of germplasm from wild relatives as gene sources except for pest resistance (Frey, 1981).

However, rice improvement through remote crosses is not always easy due to distance such as partial sterility of the hybrid and its progenies.

### **Yield Performance of Intraspecific Crosses**

Some intra- and inter-varietal crosses of four different rice ecotypes including temperate japonica ( $J_1$ ), tropical japonica ( $J_2$ ), Tongil type (T) and indica (I), generally showed the superiority to the selfed inbred parents in total dry matter, and observed and potential grain yields under the temperate environment in Suwon, Korea (Table 2). However, average harvest index did not show significant differences between inbred parental cultivar and their  $F_1$  progeny. The degree of superiority in yield potential was greater in the  $F_1$  progenies of intervarietal groups than in those of intravarietal groups, depending upon the cross combination of different varietal groups used as parents.

The tropical japonica and Tongil type varieties appeared to be useful for further increasing the yield potential. The highest mean observed yield was 966 gr/m<sup>2</sup>, which was equivalent to 9.66 t/ha in milled rice for the  $F_1$  progeny between Tongil-type and tropical japonica varieties, and was followed by the intravarietal group crosses of tropical japonica varieties. The yield advantage of the highest intervarietal  $F_1$  progeny of (T/ $J_2$ ) was 20% higher than the highest  $F_1$  progeny of tropical japonica ( $J_2$ / $J_2$ ) among the intravarietal group crosses. When it was estimated by compensating hybrid sterility based on the fertility of inbred homozygous check cultivars, the potential yield ranged 7.80 to 8.58 t/ha for inbred parental cultivars, 8.07 to 14.26 t/ha for intravarietal  $F_1$  progenies, and 9.68 to 12.31 t/ha for intervarietal  $F_1$  progenies in four different rice ecotypes. In general, the large differences between observed and potential yield may be primarily due to low hybrid fertility in the remote crosses.

The longer growth period of rice genotypes has also reduced the spikelet fertility, particularly in the temperate climates like Korea. If hybrid sterility is a problem in the crosses between different rice varietal groups, japonica and indica, it could be overcome by using the wide compatibility genes (Ikehashi and Araki, 1984). Yuan et al. (1989) also indicated that the intervarietal hybrid between indica and japonica cultivars can increase the yield potential beyond the level manifested in the intravarietal crosses of japonica or indica hybrids developed in China.

### **Yield Components and Other Characters**

In the crosses of different rice varietal groups within cultivated species, the number of spikelets per panicle and 1,000-grain weight were superior to the inbred parents (Table 3). The degree of superiority was generally greater in the intervarietal

F<sub>1</sub> progenies than in the intravarietal ones. Crosses involving tropical japonica varieties showed better performances in grain yield. The panicle numbers per plant were not significantly different between the inbred parents and the F<sub>1</sub> progenies of different varietal groups. However, the tendency towards longer panicles for the crosses, more spikelets per panicle and heavier 1,000-grain weight is expected to produce a desirable characteristics in terms of grain quality and equal distribution of nutrition of plants.

The potential grain yield of intraspecific F<sub>1</sub> progenies showed highly significant correlation with the number of spikelets per panicle (0.8267\*\*) and 1,000-grain weight (0.4473\*\*). When we looked at the genotypic distributions, the potential yield of intervarietal group F<sub>1</sub> progenies were generally higher, with more spikelets per panicle and heavier grain weights than those of the intravarietal group and inbred parents.

The observed yields were always higher with higher grain fertilities in both intervarietal and intravarietal group crosses, but it was not true for the potential yields which were derived from compensating for hybrid sterility based on mean fertility of the inbred parents. These results strongly suggest that hybrid sterility is one of the major constraints to yield improvement using remote crosses in rice.

Plant height may be another constraint to rice improvement through remote crosses with intervarietal groups, particularly with tropical japonica germplasm which is generally tall and have thick stems as well as a long panicle. Culms in the intervarietal hybrids of different varietal groups were usually longer than that of the inbred parents. Heterosis in plant height generally cause lodging, implying the importance of parental selection in the remote cross breeding programs. In this case, it was necessary to introduce semi-dwarfism in the program.

### Wide Hybridization with Wild Relatives in Korea

Wild species have been successfully used for the improvement of major crops such as rice, wheat, barley, and tomato (Brar and Khush, 1986). In Korea, the wide hybridization program started 10 years ago, with the objective of transferring useful traits from the wild *Oryza* species into the leading Korean leading varieties. It is hoped that the successful transfer of resistance to diseases (blast, bacterial blight), insects (BPH, WBPH), and tolerance to the environmental stresses (drought, cold) from the wild species can be made and will broaden the genetic base, and overcome the major constraints to the yield stability for high-yielding Japonica cultivars in Korea. For those purposes, we followed the general approaches of the wide hybridization: 1) production of interspecific hybrids between Korean elite varieties and wild species through embryo/ovule culture, 2) consecutive backcrossing with the recurrent rice parents followed by embryo/ovule culture to produce fertile introgressions, 3) evaluation of backcrossed progenies for the transfer of useful traits including diseases and insects resistance and other agronomic characters, and 4) utilization of useful introgressions as intermediate crossing parents of breeding program.

To date, hybrids have been made with several accessions of wild species belonging to AA, CC, BBCC, CCDD, and EE genomes. Several rice cultivars were used as females and wild accessions as males. Interspecific hybridization with those species of wild genomes different from *O. sativa* is hindered by cross incompatibility, resulting in the degeneration of embryos at an early stage of development, while hybridization with wild species having A genomes are achieved relatively easily. Embryo/ovule culture and application of plant growth hormones after pollination proved to be a quite effective technique to develop the interspecific hybrids. Using these two techniques, the crossabilities - the mean percentage of plant regeneration over spikelets pollinated - ranged from 4.1 to 54.6% with the highest ratio in the cross of *O. glaberrima* and the lowest in *O. australiensis*.

However, to obtain BC<sub>1</sub> and BC<sub>2</sub> plants from the hybrids between rice and wild species of other than AA genome were quite difficult due to the extremely low crossabilities caused by severe grain shattering and poor development of embryos. During the last four years, we have concentrated backcrossing on two interspecific hybrids derived from *O. sativa* and the two wild species, *O. minuta* (BBCC) and *O. grandiglumis* (CCDD). A total of nine and five BC<sub>1</sub> plants were obtained respectively from each hybrid with the crossability of 0.2%. The application of plant growth hormones after pollination did not improve grain shattering and embryo development. However, it was found that nonclipping and successive pollination together with hormone treatment significantly improved grain shattering and seed setting and thereby plant recovery was higher than the usual clipping method. We also attempted to get amphidiploids from hybrids by colchicine treatment but were not successful.

Currently advanced backcross progenies from the cross of *O. sativa* x *O. minuta* and *O. sativa* x *O. grandiglumis* are being produced, and evaluated. Backcross plants grow vigorously and have various morphological traits of the wild type, such as plant type, maturity, purple stigma, etc. Generally resistance to insect and diseases such as BPH and blast persisted upto BC<sub>1</sub> or BC<sub>2</sub> generation. However these plants were completely sterile and should be backcrossed to the recurrent parent. In the BC<sub>3</sub> generation, plants with resistant reaction to blast and BPH were produced from the cross of *O. sativa* x *O. minuta* (Table 4). Presently introgression population derived from fertile BC<sub>3</sub> or BC<sub>4</sub> plants are being evaluated for agronomic characters. Generally, this introgressed populations are similar to the recurrent parent in plant type and do not



show the undesirable traits of wild species. Some introgressed lines show segregants which possess additional chromosomes of wild species. Some lines are heading quite early, suggesting that useful transgressive variants might be created through the wide hybridization between different genomes.

So far, the progress in wide hybridization was quite slow mainly due to low efficiency of production of backcross progenies. Therefore more intensive efforts are needed to develop elite introgression lines with useful traits of wild species. In the future, to detect alien genetic variation and to tag introgressed alien chromosome segments should be done with these materials.

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**Table 1. Yield of indica/japonica (I/J) and japonica (J) cultivars in Korea, 1974 - 1991.**

Year	Area			Average yield (t/ha)		Yield advantage of I/J (%)	Contribution of I/J to rice production (%)
	Total	I/J	J	I/J	J		
	(million ha)	(%)	(%)				
1974	1204	15.2	84.8	4.73	3.53	34.0	19.4
1975	1218	22.9	77.1	5.03	3.51	43.0	29.8
1976	1214	44.6	55.4	4.79	3.96	21.0	49.3
1977	1230	54.6	45.4	5.53	4.23	30.7	61.2
1978	1230	76.2	23.8	4.86	4.35	11.7	78.1
1979	1233	60.8	39.2	4.63	4.37	5.9	62.2
1980	1233	49.5	50.5	2.87	2.92	-2.7	49.1
1981	1224	26.5	73.5	4.37	4.08	7.1	27.8
1982	1188	32.9	67.1	4.89	4.13	18.4	36.7
1983	1228	34.3	65.7	4.83	4.20	15.0	37.5
1984	1230	30.0	70.0	5.02	4.46	12.6	32.5
1985	1237	27.8	72.8	5.04	4.37	15.3	30.8
1986	1236	22.1	77.9	4.72	4.49	5.1	23.0
1987	1262	19.6	80.4	4.57	4.31	6.0	20.6
1988	1260	17.9	82.1	5.36	4.69	14.3	19.9
1989	1257	14.5	85.5	5.11	4.63	10.4	15.8
1990	1244	11.2	88.8	5.20	4.42	17.6	12.9
1991	1208	4.1	95.6	4.94	4.46	10.8	4.5

**Table 2. Comparative yield performance of parental types and their hybrids in intra-and intervarietal group crosses, Suwon, Korea.**

Parental type/hybrids	Sample size	Total dry matter g/m <sup>2</sup>	Harvest index %	Grain yield (milled) <sup>1</sup>	
				Observed -----g/m <sup>2</sup> -----	Potential
Inbreds (mean)	13	1875	48	636	815
Indica (1)	3	1707 dg	45 a	550 fg	843 dg
Temperate					
Japonica (J <sub>1</sub> )	3	1734 dg	53 a	660 de	823 eg
Tropical					
Japonica (J <sub>2</sub> )	2	2252 b	45 a	704 bd	858 dg
Tongil (T)	5	1910 cf	47 a	646 de	780 fg
Hybrids (mean)	37	2138	42	647	1025
Intervarietal (mean)	10	1928	46	618	970
I/I	2	1800 dg	45 a	577 cg	1079 bc
J <sub>1</sub> /J <sub>1</sub>	3	1634 g	54 a	628 dc	807 g
J <sub>2</sub> /J <sub>2</sub>	1	2935 a	35 a	804 b	1426 a
T/T	4	1960 cd	44 a	602 ef	924 cf
Intervarietal (mean)	27	2414	41	650	1046
I/J <sub>1</sub>	3	1824 e	36 a	461 h	968 ce
I/J <sub>2</sub>	4	2607 a	40 a	734 bc	1202 b
T/J <sub>2</sub>	4	2888 a	46 a	966 a	1231 b
I/T	8	2096 bc	46 a	696 cd	973 cd
J <sub>1</sub> /T	8	1948 ce	36 a	510 gh	977 cd

<sup>1</sup> Figures marked with different letters are significantly different at the 5% level using DMRT. Potential yield was calculated by multiplying the following components: no of panicle, no. of spikelets, 1000-grain weight, mean fertility of inbred check varieties.

**Table 3. Comparative production traits performance of parental types and their hybrids in intravarietal and intervarietal crosses, Suwon, Korea.**

Parental type, hybrid	Sample size	Days to head	Culm cm	Panicle		Grains /pan.	1000 grs. wt. gr.	Fertility %
				Length cm	No.			
<b>Inbreds</b>								
Indica (I)	3	123	69	26	18	106	23.2	65
Japonica (J <sub>1</sub> )	3	112	73	20	19	140	22.6	83
Japonica (J <sub>2</sub> )	2	120	126	28	11	204	30.3	88
Tongil (T)	5	110	68	23	16	147	23.9	83
<b>Hybrids</b>								
Intravarietal								
I/I	2	127	82	29	17	193	24.6	68
J <sub>1</sub> /J <sub>1</sub>	3	107	73	21	17	151	23.3	81
J <sub>2</sub> /J <sub>2</sub>	1	129	141	31	13	254	31.0	74
T/T	4	116	75	24	16	182	23.8	73
Intervarietal								
I/J <sub>1</sub>	3	115	104	26	16	167	26.9	57
I/J <sub>2</sub>	4	119	127	30	14	213	31.9	73
I/T	4	120	78	27	15	182	25.7	83
T/J <sub>2</sub>	8	111	124	27	14	224	28.3	90
J <sub>1</sub> /T	8	112		25	16	184	24.4	55

**Table 4 . Production of hybrids and backcross progenies and their reaction to blast and BPH in the wide hybridization with *O. minuta*.**

Material	Plants	No. of plants with resistant reaction		
		Blast		BPH/WBPH
		KJ 301	KI313	
Hwaseongbyeo	8	0	0	0
<i>O. minuta</i> (Acc. 101144)	8	8	8	8
F <sub>1</sub>	10	10	10	10
BC <sub>1</sub>	8	8	8	7
BC <sub>2</sub>	4	3	3	3
BC <sub>3</sub>	32	7	3	4

## RICE GERMPLASM ENHANCEMENT IN JAPAN

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### INTRODUCTION

Plant genetic resources play an important role in crop improvement programs. Landraces are adapted to local environments and can furnish useful traits to broaden the genetic base of crops. Wild relatives of crop species also have a potential for plant breeding as genetic resources, particularly for pest and disease resistance and tolerance to environmental stresses. These plant genetic resources, however, are now gradually disappearing, a process known as genetic erosion. The reasons for this include the widespread use of improved varieties, socio-economic changes in agriculture and rapid urbanization. The loss of genetic diversity implies that diverse genes are being lost. Due to genetic erosion worldwide and increasing genetic uniformity, there are increasing concerns to conserve genetic diversity for future use.

The Ministry of Agriculture, Forestry and Fisheries (MAFF) in Japan has conducted a nationwide project, which is called the "MAFF Genebank Project," to conserve genetic diversity since 1983. Development of the genebank system has been accelerated to solve urgent problems in the overall management of genetic resources. The first 10-year plan of the MAFF Genebank Project included not only plants but also microorganisms, animals, forest trees, and aquatic organisms. The National Institute of Agrobiological Resources (NIAR) is the central organization in Japan for plant, microorganism and animal conservation. The Genetic Resources Center at NIAR is in this way playing a uniting role in close cooperation with co-working national and international research organizations. In 1993, 10 years after the beginning of the project, the project was reconstructed and entered into a second stage for all the areas of genetic resources. In 1994, DNA and molecular genetic information was added as a new category of the MAFF genebank system, in association with the Rice Genome Research Program (RGP) which has been organized by NIAR.

#### **Efforts to Conserve Rice Genetic Resources in Japan**

Rice is the crop with the most accessions stored in the National Genebank at NIAR and about 28,000 accessions are conserved. A national program of comprehensively collecting rice germplasm throughout Japan was conducted between 1962 and 1965. More than 1,300 local varieties were collected from 27 prefectures in Japan (Omura, 1970). A total of 21 collecting missions during four years (1962-1965) were undertaken to conserve indigenous rice varieties before they disappeared. After completing the project to collect rice varieties in Japan, exploration missions for collecting rice genetic resources have been mainly directed abroad.

Since 1977, Japanese scientists have taken part in overseas explorations with the scientists in collaborating countries. During these missions rice germplasm has been one of the main target species for collecting (Table 1). Since 1994 collaborative exploration between Japanese and Vietnamese scientists has contributed to conservation of rice germplasm in Vietnam. Northern Vietnam is in the subtropical and tropical zones, and is included in the center of diversity of Asian cultivated rice, *Oryza sativa* L. Rice is the principal crop and is grown under upland, rainfed, and irrigated conditions in this region. Almost all rice landraces grown under irrigated condition are now improved varieties. The majority of the approximately 1,000 rice varieties collected are upland rice, which are grown by Vietnam's minority ethnic groups. Isozyme and DNA polymorphism showed that traditional rice varieties in northern Vietnam remained diverse (Okuno et al., unpublished). These germplasm collections are conserved in both countries.

More than 800 rice germplasm were introduced from Yunnan province, China, through a 10-year collaborative breeding program to develop improved varieties adapted to Yunnan province. A part of the rice germplasm introduced from this province has been selected as promising breeding materials with tolerance to cold injury and has been used in rice breeding in Japan. In 1992, the MAFF and International Rice Research Institute (IRRI) agreed that the National Genebank based at NIAR would be a duplicate storage facility for some of the rice germplasm in the IRRI collection. Since then, annually

about 200 accessions of rice have been introduced from IRRI. Similarly, rice germplasm collected by Japanese scientists during collaborative overseas missions is sent to IRRI for duplicate storage.

NIAR has the job of growing rice germplasm introduced under quarantine rules as well as basic characterization of the germplasm introduced. The Genebank System has systematically characterized, evaluated, and preserved rice germplasm. The seed samples multiplied are distributed to rice scientists and breeders. The passport data and basic characteristics of rice germplasm will be available on the Internet soon.

The Ministry of Education in Japan also conserves plant genetic resources. Much research by scientists at the National Institute of Genetics, Japan, has focused on population and ecological genetics of wild relatives of rice having the AA genome. Study tours to Asia, Africa, and Latin America have shed new light on this complex group of species (Oka, 1988). The first comprehensive ecogeographic information on wild rice in the central Amazon basin, Brazil, was reported (Morishima et al., 1994). Through these works, wild relatives of rice have been collected and preserved at the National Institute of Genetics.

### **Germplasm Enhancement using Introduced Rice Varieties**

While indigenous Japanese varieties have been the main source of germplasm for rice improvement in Japan, introduced varieties have been used to supply special traits. Pest and disease sources, and cold tolerance at the booting stage from foreign countries have been incorporated into Japanese varieties. Recently, a lipoxygenase-3 deficient variety introduced from Thailand was found as a gene source to improve the storage quality of rice. To date, nine varieties from outside Japan were used to develop new lines registered by the Japanese MAFF (Table 2).

#### **(1) Disease resistance**

The major disease facing Japanese rice farmers is blast and good sources of resistance against rice blast disease have been sought. However, most of them have proved to involve major genes that can easily be overcome. In 1960, Kusabue was released using a Chinese gene source for blast resistance, *Pik*, but the resistance in this variety broke down after only two years. Tadukan from the Philippines, Zenith from the United States and Te Tep from Vietnam were also used as sources of resistance to blast, but in these cases the resistance has not held up in Japanese conditions. To overcome the rapid breakdown of blast resistance, multilines with different major resistance genes have been produced with the genetic background of different Japanese varieties. Sources of durable resistance to blast continue to be sought in the germplasm collection. Since Kusabue broke down soon after its release to farmers, rice breeders in Japan have paid attention to field resistance to blast, which is controlled by multiple genes. Although Japanese upland rices are good sources of field resistance, these varieties have several undesirable characteristics for irrigated rice development and the genetic base of field resistance has not been identified.

Bacterial leaf blight (BLB) is also a major disease in Japan. Collaborative work between MAFF and IRRI has resulted in a large number of resistance genes to this disease being identified. Now breeders have over 20 genes for resistance to BLB available to cope with this disease.

A total of 19 varieties with resistance to stripe virus have been developed in Japan. The resistance of 14 varieties out of 19 is derived from the same source, the Indian variety Modan. This resistance gene was mapped on the short arm of chromosome 11 using DNA markers (Hayano et al., unpublished). Since resistance is derived from limited sources, there is a danger that this resistance may breakdown. A new approach to develop resistance to stripe virus has been directed to developing transgenic plants which have incorporated the gene which is responsible for the coat protein of stripe virus (Otsuki, 1993).

#### **(2) Insect resistance**

Brown planthopper (BPH) is an important insect pest in Japan. BPH resistance genes, *Bph1*, *bph2*, *Bph3*, and *bph4*, have been incorporated into Japanese varieties from the varieties, Mudgo from India, Babawee and Rathu Heenati from Sri Lanka, and IR1154-243 from IRRI. Some breeding lines with each of these resistance genes have been developed, but no commercial resistant varieties have yet been released in Japan.

Resistance to the green leafhopper (GLH), which is associated with the tungro virus, has been found in the Chinese variety Pe-bin-hun and the Indian varieties C203-1 and Lepe dumai. QTL analysis indicated that the resistance to GLH of Lepe dumai was controlled by a set of complementary genes on chromosomes 3 and 11 (Tamura et al., 1996). Other sources of GLH resistance have been found, but these have not been registered with MAFF.

#### **(3) Cold tolerance**

The Japanese rice crop sometimes experiences severe cold injury. In the history of rice production in Japan cold injury has been the principal cause of major crop loss. There are two types of cold injury. The first type results in sterility due to low temperatures, of below 17°F during the booting stage and the time of heading. In the second type, low temperature

during vegetative growth results in delayed heading and maturity, and finally leads to a decrease in yield associated with defective grain filling.

Cold tolerance during the reproductive growth stage is important for rice production and a major objective in the rice breeding program of northern Japan. Cold tolerance at the booting stage was found in the Indonesian variety Silewah (Satake and Toriyama, 1979) and the Malaysian variety Padi Labou Alumbis. Two genes confer cold resistance in Silewah were detected and linked with RFLP markers at the end of chromosomes 3 and 4 (Saito et al., 1995).

#### **(4) Storage quality**

Deterioration of rice grains and generation of stale flavor during storage are serious problems, which reduce the quality of stored rice grains. The degradation of lipids causes these changes during storage (Yasumatsu and Moritaka, 1964; Yasumatsu et al., 1966; Aibara et al., 1986). Lipoxygenase (LOX) catalyzes the incorporation of molecular oxygen into fatty acids to form hydroperoxides and is related to the formation of volatile compounds. The deficiency of LOX enzyme in rice grains may alleviate oxidative deterioration. Three isozymes of LOX have been detected in rice embryos and LOX-3 is the major component of the isozymes accounting for 80 to 90% of total LOX activity (Ida et al., 1983).

Monoclonal antibodies against LOX-3 from rice embryos were prepared to specially detect the LOX-3 isozyme (Suzuki et al., 1992). Using these monoclonal antibodies, we screened for LOX-3 deficiency. The Thai variety, Daw Dam, from the rice germplasm collection was found to be deficient for LOX-3. The deficiency of LOX-3 in this variety is controlled by a single recessive gene *lox3* (Suzuki et al., 1996). Daw Dam belongs to the tropical japonica rice group. Incorporation of the null allele into Japanese lines/varieties with superior agronomic background is currently underway.

### **Rice Genome Research in Japan**

In 1991, the Rice Genome Research Program (RGP) was initiated by MAFF to analyze the molecular basis of the rice genome organization and function. The first stage of this program organized by NIAR was conducted from 1991 to 1997 and consisted of three main projects, 1) construction of a genetic linkage map using DNA markers, 2) construction of a physical map of rice chromosomes, and 3) large-scale sequencing and cataloging of cDNA from several organs of the rice plant.

#### **(1) Construction of a genetic linkage map**

McCouch et al. (1988) and Saito et al. (1991) constructed the first linkage map involving DNA markers in rice. During the first stage of the RGP, emphasis was placed on the construction of a high density and high-resolution linkage map. F<sub>2</sub> plants, which were derived from the cross between a japonica variety, Nipponbare, and an indica variety, Kasalath, were used to develop the linkage map. To date, a linkage map with about 2,500 DNA markers has been produced from this cross. DNA markers consist of cDNA fragments derived from callus, root, and shoot; genomic DNA fragments and RAPDs mapped on 12 rice chromosomes (Harushima et al., 1998). These DNA markers cover about 1,500 cm of genetic distance. It is noteworthy that all the cDNA fragments and most of the genomic fragments were partially sequenced to convert them into STS (sequence tagged site) markers (Inoue et al., 1994). These DNA markers are useful for tagging genes, which are responsible for biological and agronomic traits.

Genomic DNA fragments of wheat have been mapped on the rice linkage map. The synteny analysis of these plants revealed a high conservation in the order of DNA markers (Kurata et al., 1994). The synteny map and the mapped rice probes will be useful for the molecular analysis of the corresponding chromosomal regions in cereal crops.

#### **(2) Construction of a physical map**

Densely distributed DNA markers on the linkage map enable construction of a physical map of the rice genome. A yeast artificial chromosome (YAC) library of large DNA fragments has been constructed. A total of about 2,400 YAC clones have been ordered along the linkage map of rice chromosomes. These clones can be used for map-based cloning of genes having agronomic value.

#### **(3) Large-scale cDNA analysis**

Large-scale cDNA analysis aims at cataloging all the expressed genes in different organs of rice (Sasaki et al., 1994). About 15,000 cDNA clones have been partially sequenced. The function of sequenced cDNA clones was deduced by similarity search to the protein database. Only 25% of the cDNA clones analyzed showed a similarity to known proteins, suggesting that most of the expressed genes encode for unknown proteins.

#### **(4) The second stage of RGP**

Since 1998, the RGP has entered a new stage focusing on large-scale sequencing of the rice genome, QTL analysis of agronomically important traits, cloning and characterization of genes, and marker-based selection in rice breeding. The

program also includes the development of mutant lines, which are tagged by retrotransposons of rice as a strategy for gene isolation.

### Researches on Rice Germplasm Enhancement Using DNA Markers

One of the uses of DNA markers in germplasm enhancement is the analysis of the linkage relationship between DNA markers and genes for agronomic traits. Using DNA markers, we have evaluated rice germplasm for hybrid breakdown, which is one of the reproductive barriers found in indica/japonica rice crosses, field resistance to rice blast of Japanese upland varieties and mesocotyl elongation. We report here the recent results obtained from genetic studies of these characters using DNA markers to improve Japanese rice varieties.

#### (1) Hybrid breakdown

Hybrid breakdown, a reproductive barrier in cultivated rice, was found in the  $F_2$  of crosses between the Thai variety Col No.15 and the Japanese variety Sasanishiki. As over 30% of the  $F_2$  plants show weakness, hybrid breakdown may cause a decrease in selection efficiency during breeding. Therefore, the determination of genotypes for hybrid breakdown is required to choose desirable parental varieties in breeding program of indica/japonica rice crosses.

A pair of complementary recessive genes control hybrid breakdown in rice (Okuno, 1985; 1998). Linkage analysis and interval mapping were performed using MAPMAKER (Lander et al., 1987). Linkage between hybrid breakdown genes and RFLP markers was detected in  $F_2$  plants from the crosses between a tester line, W26, with four recessive alleles and the Indonesian variety Siborunauli 1, and between Col. No. 15 and W26 (Fukuoka et al., 1998). Interval mapping using 9 RFLP markers located near the terminal end of chromosome 10, indicated that one of complementary genes, hwd1, was located between C701 at a distance of 0.9cm and R2309 at a distance 0.6cm. Mapping of the other gene, hwd2, was conducted using 18 markers in the middle region of chromosome 7. Eight RFLP markers in this region cosegregated with hybrid breakdown and were mapped in two sites at a distance of 1.1cM.

#### (2) Field resistance to rice blast

Resistance to rice blast is of two types, true (vertical) resistance and field (horizontal) resistance. Field resistance is defined as the resistance that allows effective control of a pest under natural field conditions and is durable when exposed to new races of the pest. After observing the rapid breakdown of the true resistance gene *Pik* to blast, rice breeders have tried to incorporate field resistance into Japanese varieties. Japanese upland varieties are potential gene sources for field resistance. Linkage analysis with conventional genetic markers has indicated several different loci conferring field resistance to blast in Japanese upland rice (Higashi and Saito, 1985).

We have studied the chromosomal location of genes controlling field resistance to blast in Japanese upland rice using RFLP markers (Fukuoka and Okuno, 1997). A total of 146  $F_4$  progeny from the cross between Nipponbare (moderately susceptible, irrigated) and Owarihatamochi (resistant, upland) were used for QTL analysis. More than 40% of RFLP markers on the rice linkage map showed polymorphism between these two varieties. As a result, five QTL at a LOD threshold of 2.0 were detected on chromosomes 2, 4 (2 loci), 9, and 12 (Table 3). Four QTLs located on chromosomes 2, 4, and 12 were derived from Owarihatamochi. QTL which was linked to a RFLP marker, G271, in the middle of chromosome 4 held 47.8% of the phenotypic variance for resistance to blast. Another QTL on chromosome 4 held 31.0% of the variance for the resistance. The loci linked to RFLP markers chromosome 4 played a major role in the expression of field resistance, confirming the results of an earlier study (Wang et al., 1994).

Resistant  $BC_1F_2$  plants occurred in the progeny of  $BC_1F_1$  with either of QTLs on chromosomes 2, 4, or 12 suggesting that resistance to blast of upland rice have been incorporated into the recurrent parental variety by RFLP marker-based selection. One of QTLs showed a Mendelian inheritance, suggesting that one of the field resistance genes to blast is controlled by a single gene.

#### (3) Mesocotyl elongation ability

The mesocotyl, between the seminal root and coleoptile, elongates in the dark and is related to emergence of rice seeds. The degree of mesocotyl elongation is one of the traits associated with stand establishment in direct-seeded rice. QTL analysis of loci conferring mesocotyl elongation ability was undertaken in rice using RFLP markers.  $F_3$  progeny lines of the cross between Surjamkhi (39.2mm) and Dao Ren Qiao (0.0mm) were used for QTL analysis. Three QTLs at a LOD threshold of 2.0 were detected on chromosomes 3, 6, and 11 (Table 4, Katsuta et al., 1996). One of QTLs was linked to a RFLP marker near the end of chromosome 3 and held 32.3% of the phenotypic variance for mesocotyl elongation ability. The progeny has been backcrossed to the Japanese variety Kinuhikari.



## CONCLUSIONS

The Rice Genome Program (RGP) has had a great impact on both rice genetics and rice breeding, resulting in a broadened use of the rice germplasm collection. High-density linkage maps and DNA markers, which are distributed over 12 rice chromosomes, are very powerful tools in germplasm evaluation and enhancement. Yano et al. (1997) reported that these products supplied by the RGP have contributed much to the genetic dissection of QTLs conditioning heading time of indica and japonica rice varieties. In addition to DNA marker-based selection of agronomic traits, each of the QTLs responsible for agronomically important traits can be tagged by DNA markers and can be isolated by map-based gene cloning. These research highlights of the RGP will also rapidly affect the progress in evaluation and enhancement of the rice germplasm collection not just in Japan but worldwide.

Since the establishment of the DNA bank in 1994, DNA clones produced by the RGP have been furnished to scientists worldwide. The Genome Information Center will be constructed in early 1999 to cope with large-scale sequencing of the rice genome.

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**Table 1. Collaborative missions to collect rice genetic resources.**

Year	Country	No. of samples collected
1985	Nepal	293
1986	Thailand	113
1988	Indonesia	209
1989	Pakistan	191
1990	Thailand	210
1991	Pakistan	58
1993	Madagascar	123
1994	Vietnam	219+189
1995	Vietnam	153+131
1996	Vietnam	153
1997	Vietnam	119

**Table 2. Registered genetic stocks using rice genetic resources from outside Japan.**

Registration	Year	Trait	Source	Origin
No. 2	1984	GLH	Pe-bi-bun	China
No. 3	1984	BPH	Mudgo	India
No. 4	1985	BPH	IR1154-243	Philippines
No. 5	1985	GLH	C203-1	India
No. 6	1986	GLH	Lepedumai	India
No. 7	1987	BPH	Babawee	Sri Lanka
No. 8	1987	Cold tolerance	Silewah	Indonesia
No. 10	1988	BPH	Rathu Heenati	Sri Lanka
No. 11	1990	Cold tolerance	Padi Labou Alumbis	Malaysia

**Table 3. Markers linked to QTLs conferring field resistance to rice blast.**

Markers linked	Chromosomes	Origin of resistance <sup>1</sup>	LOD <sup>2</sup>	% Variation explained <sup>2</sup>
G271	4	O	20.5	47.8
Y8026L	4	O	11.7	31.0
G2140	12	O	4.5	13.3
C777	2	O	2.0	6.3
G103	9	N	2.0	6.2

<sup>1</sup> O and N indicate Owarihatamochi and Nipponbare, respectively.

<sup>2</sup> LOD and percent variation explained were calculated at the putative QTLs by MAPMAKER/QTL (Lander and Botstein, 1989).

**Table 4. Markers linked to QTLs conferring mesocotyl elongation ability.**

Markers linked	Chromosomes	Distance to QTLs (cM)	LOD <sup>1</sup>	% variation explained <sup>1</sup>
G175	3	9.0	8.1	32.3
G1091	6	0.0	2.6	11.7
G320	11	14.0	2.4	10.7

<sup>1</sup>LOD and percent variation explained were calculated at the putative QTL's MAPMAKER/QTL. (Lander and Botstein, 1989).

# MARKER-ASSISTED DISCOVERY AND TRANSFER OF BENEFICIAL QUANTITATIVE TRAIT LOCI ALLELES FROM THE WILD RICE RELATIVE *Oryza rufipogon*.

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## ABSTRACT

A marker-assisted advanced backcross breeding strategy was employed to broaden the germplasm base of cultivated rice by simultaneously detecting and transferring yield-enhancing QTL alleles from the wild rice relative *Oryza rufipogon* into elite rice varieties. The advanced backcross QTL strategy uncovers positive alleles which otherwise would be overlooked based on the phenotype, facilitates the use of exotic germplasm, and permits rapid transfer of valuable alleles into cultivars. Multiple advanced-backcross populations were developed, beginning with a cross between the Chinese hybrid variety V20/Ce64 and *O. rufipogon*. The QTL analysis from this population confirmed the presence of trait-improving QTL alleles from *O. rufipogon*, in spite of the inferior phenotype (Xiao et al., 1998). QTL analyses for four additional interspecific populations have either been completed or are approaching completion, providing additional confirmation of the presence of beneficial QTL alleles in wild rice relatives. Multiple studies using *O. rufipogon* as the donor parent also provides the opportunity for comparisons of wild QTL alleles across environments and in different genetic backgrounds. Preliminary results reveal trait-improving wild QTLs shared across studies, forming the basis for further characterization of these QTLs and transfer into elite cultivars.

## INTRODUCTION

The domestication process and intensive breeding efforts have contributed to a narrowing of the gene pool of modern rice cultivars. Consequently, wild and unadapted germplasm represents the majority of the existing genetic variation in rice. However, wild germplasm is generally not considered useful for improving key quantitative traits of agronomic importance, such as yield, because of the overall inferior performance relative to cultivated species. The advent of molecular markers and maps, however, make it possible to identify individual quantitative trait loci associated with beneficial traits such as yield, quality, and disease resistance (Tanksley, 1993; McCouch and Doerge, 1995). One recent strategy proposed to identify positive alleles in an otherwise undesirable background is the advanced backcross QTL method (Tanksley and Nelson, 1996). This method, first demonstrated in tomato, uses marker-assisted QTL detection to locate trait-enhancing alleles in a variety, independent of phenotypic selection (Tanksley et al., 1996). In addition, by using the advanced backcross population structure, which delays the QTL analysis until the BC<sub>2</sub> or BC<sub>3</sub> generation, valuable QTLs identified can be rapidly transferred into elite breeding lines. Here we report the progress of multiple molecular marker-facilitated studies in rice in determining the potential for using wild relatives to improve key quantitative traits of agronomic importance in elite cultivars.

## PROCEDURES

The wild rice species used as donor parents were chosen based on the degree of genetic distance between the wild and cultivated gene pools, as well as their ability to hybridize to cultivars through sexual crossing. As described in Xiao et al.

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(1998), accessions were obtained from the International Rice Germplasm Collection (IRGC) at the International Rice Research Institute (IRRI), representing the seven AA genome species. Thirty-four of these accessions, along with 15 accessions from *O. sativa*, were probed with 25 RFLP markers distributed on the 12 chromosomes of rice to determine the degree of genetic distance between the wild and the cultivated gene pools. Three wild rice relatives were chosen to be donor parents in the advanced backcross strategy: *O. rufipogon* (IRGC 105491), *O. glaberrima* (IRGC 103544), and *O. barthii* (IRGC 104119).

Advanced interspecific backcross populations were then developed using these wild rice relatives, with priority given to *O. rufipogon* for the initial studies, followed by *O. glaberrima* and *O. barthii*. We proceeded to develop, in collaboration with several international research groups, advanced backcross populations (BC<sub>2</sub> families) for *O. rufipogon* crossed with four elite cultivars (Table 1). These cultivars represent some of the highest-yielding varieties in the world, including a hybrid variety from China (V20/Ce64), a popular indica cultivar (IR64), a high quality U.S. variety (Jefferson), and an adapted upland South American variety (Caiapo). In addition, the initial population using *O. glaberrima* as the donor parent was also developed. Other populations currently in progress include additional crosses with *O. glaberrima*, as well as crosses with *O. barthii*.

Field trials of the BC<sub>2</sub> families were conducted for each population in the adapted environment of each recurrent parent. Primary agronomic traits, such as grain yield and yield-related traits, including panicle length, panicles per plant, grains per panicle, grains per plant, spikelet fertility, and 1000-grain weight, were measured on all populations. In addition, grain quality characteristics, negative traits such as shattering and dormancy, and disease resistance were included when possible. Three of the five studies analyzed to date have field data from multiple environments, with population sizes ranging from 258 to 353 BC<sub>2</sub> families. QTL analyses are performed using both RFLP markers (Causse et al., 1994) and microsatellite markers (Chen et al., 1997) as previously described in McCouch et al. (1988) and Panaud et al. (1996). Approximately 130 markers are genotyped for each population, with emphasis given in the later populations for shared markers between studies to aid in comparisons. Statistical analyses are performed using qGene (Nelson, 1997).

Subsequent to the QTL analyses, near-isogenic line development is initiated to isolate trait-enhancing QTLs in the recurrent parent background for further characterization and to lead to the development of improved hybrid and inbred varieties. An ambitious NIL development program begun in 1996 has steadily progressed lines targeting two yield-enhancing QTL from *O. rufipogon*, yld1.2 and yld2.1, to improve elite hybrid varieties by transferring the QTLs into three restorer lines (Jiming Li, personal communication; Fig. 1). These lines are currently undergoing continued marker selection, in addition to phenotypic selection, and will be tested in yield trials in China in 1999. Near-isogenic line development has also begun with the Jefferson/*O. rufipogon* population in order to develop the genetic materials necessary for molecular characterization of wild QTLs.

## RESULTS AND DISCUSSION

The first QTL analysis completed was the V20/Ce64 and *O. rufipogon* population. This analysis confirmed the efficacy of the advanced backcross QTL strategy in rice: while the *O. rufipogon* accession was phenotypically inferior for all 12 traits examined, transgressive segregation was seen for all traits, and 51% of the significant QTLs had beneficial alleles from *O. rufipogon* (Xiao et al., 1998). For example, seven QTL showed significant association with grain yield, with the *O. rufipogon* alleles associated with yield increases at four of these loci ranging from 0.98 to 1.22 tons per hectare over the hybrid variety V20/Ce64. Two yield-enhancing QTLs from *O. rufipogon* on chromosomes 1 and 2 (yld1.1 and yld2.1) were associated with yield increases of 18% and 17% over the hybrid variety, without delaying maturity, increasing plant height, or negatively influencing any of the traits measured so far in this population (Xiao et al., 1998). Yield QTLs corresponding to the same region as yld1.1 have also been identified in three other populations (Fig. 2).

Two other QTL analyses have also been completed: the V20/Ce64 and *O. glaberrima* population and the Caiapo/*O. rufipogon* population. These analyses showed results similar to the first, with transgressive segregation observed, and multiple beneficial alleles detected from the wild rice parent (Li et al., unpublished; Moncada et al., unpublished). Two additional QTL studies, Jefferson/*O. rufipogon* and IR64/*O. rufipogon*, are in progress (Table 1). Our strategy is uniquely positioned to answer the genetic questions concerning the action of QTLs in different backgrounds and environments, as we have used the same donor parent, *O. rufipogon*, in multiple backgrounds and environments. Preliminary comparisons between studies indicate beneficial QTL alleles from *O. rufipogon* identified in different backgrounds. For example, a QTL associated with heading date is observed in the same region on chromosome 1 in three populations, with the *O. rufipogon* allele contributing to earliness in two of these populations (Fig. 3).

The positive results from these initial studies suggest that genes introgressed from *O. rufipogon* into an elite genetic background can improve key agronomic traits of an elite rice variety, even though *O. rufipogon* itself is phenotypically inferior to the cultivated variety (Tanksley and McCouch, 1997). Yet, as evident by Ragot et al. (1995) and Tanksley et al.

(1996), exotic or wild QTL alleles that are favorable for some traits are often associated with deleterious effects on other traits. This phenomenon was observed in 16 of the 35 (46%) of the cases in the V20/Ce63 and *O. rufipogon* study. For example, *O. rufipogon* alleles at yld8.2 and yld12.1 contributed positively to grain yield, but increased plant height or grain weight, and *O. rufipogon* alleles in the genomic region around RZ730 on chromosome 1 increased panicle length, spikelets per panicle, spikelets per plant, and grains per panicle, but also increased plant height (Xiao et al., 1998). Either pleiotropy, where a single gene affects multiple characters, or tight linkage of multiple QTL, each one affecting a separate character, can be the genetic cause. In cases where linkage was the reason behind the association, marker-assisted disruption of the deleterious linkage would potentially make it possible to use these positive wild QTL alleles for genetic improvement. Fine mapping and further genetic dissection of the target regions containing these QTL would be needed to distinguish between pleiotropy and linkage of multiple genes.

These results have raised some additional questions that have yet to be answered. What proportion of QTLs from unrelated wild sources are allelic with one another? What proportion of rice QTLs are comparable to QTLs detected in maize and the Triticeae? What is the function of the genes underlying trait-improving QTL? What is the molecular genetic nature of the phenotypic differences in an allelic series of a QTL? These questions can now be studied, given the progress made from the completed wild QTL studies—including the accumulated information on the effects and locations of trait-improving wild QTLs, the genetic materials and NILs developed, in addition to the recently-available technologies improving the efficiency of phenotype-based gene cloning. The effective use of molecular maps and markers combined with QTL analysis and targeted introgression, and ultimately, cloning and transformation of genes underlying QTLs, will help lead to more effective use of rice germplasm resources, and may contribute to future yield increases.

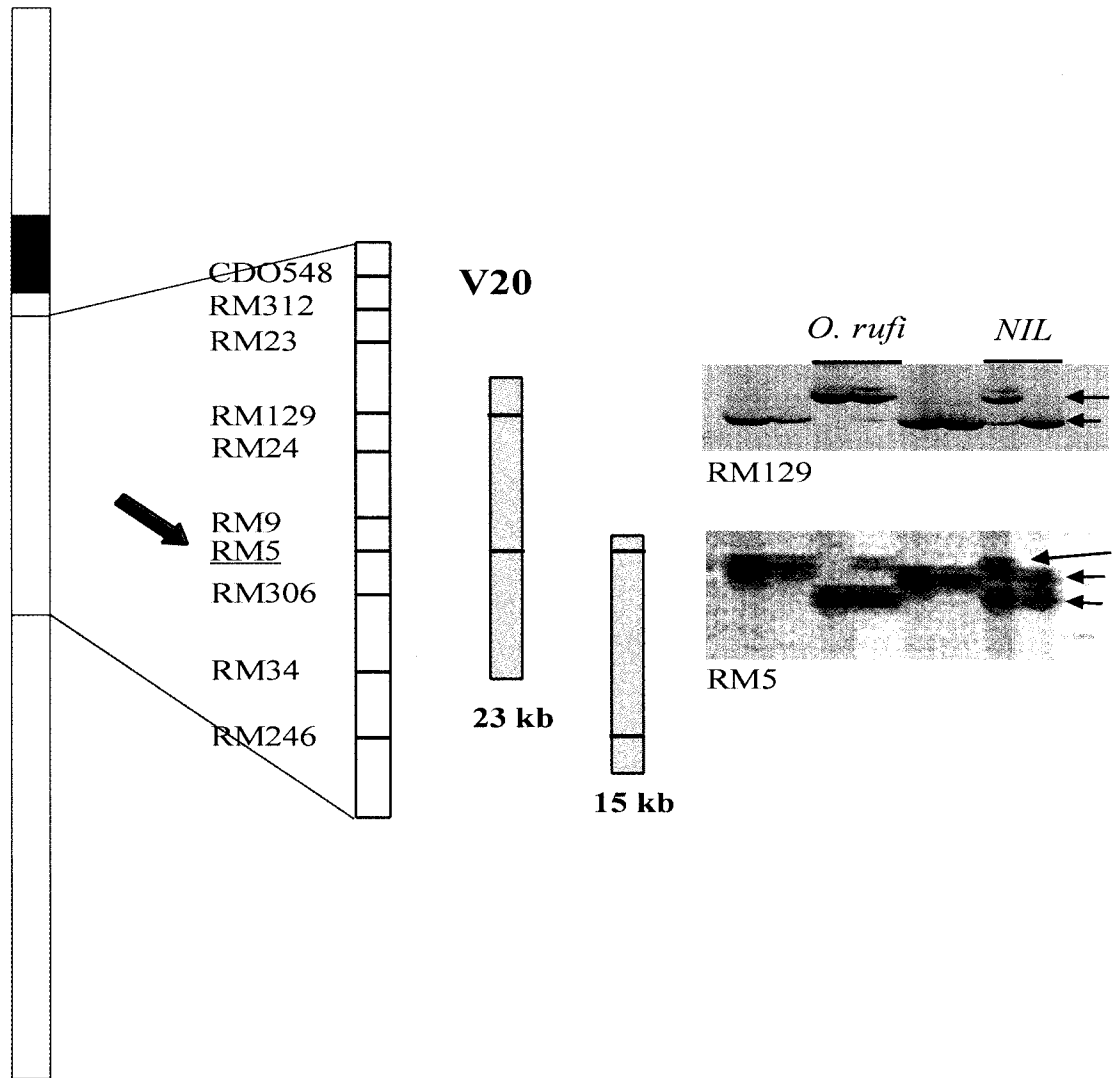
#### ACKNOWLEDGMENTS

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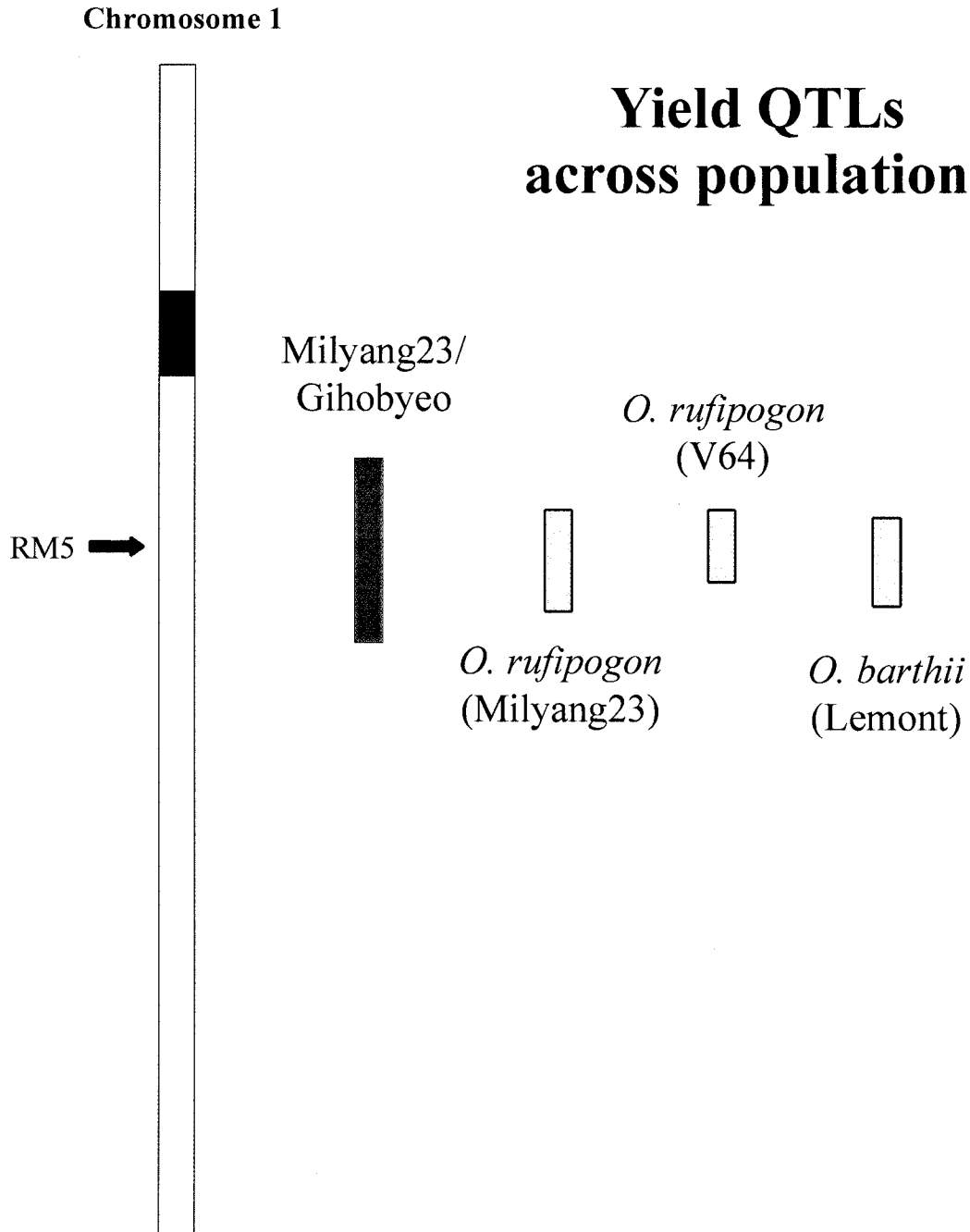
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**Chromosome 1**

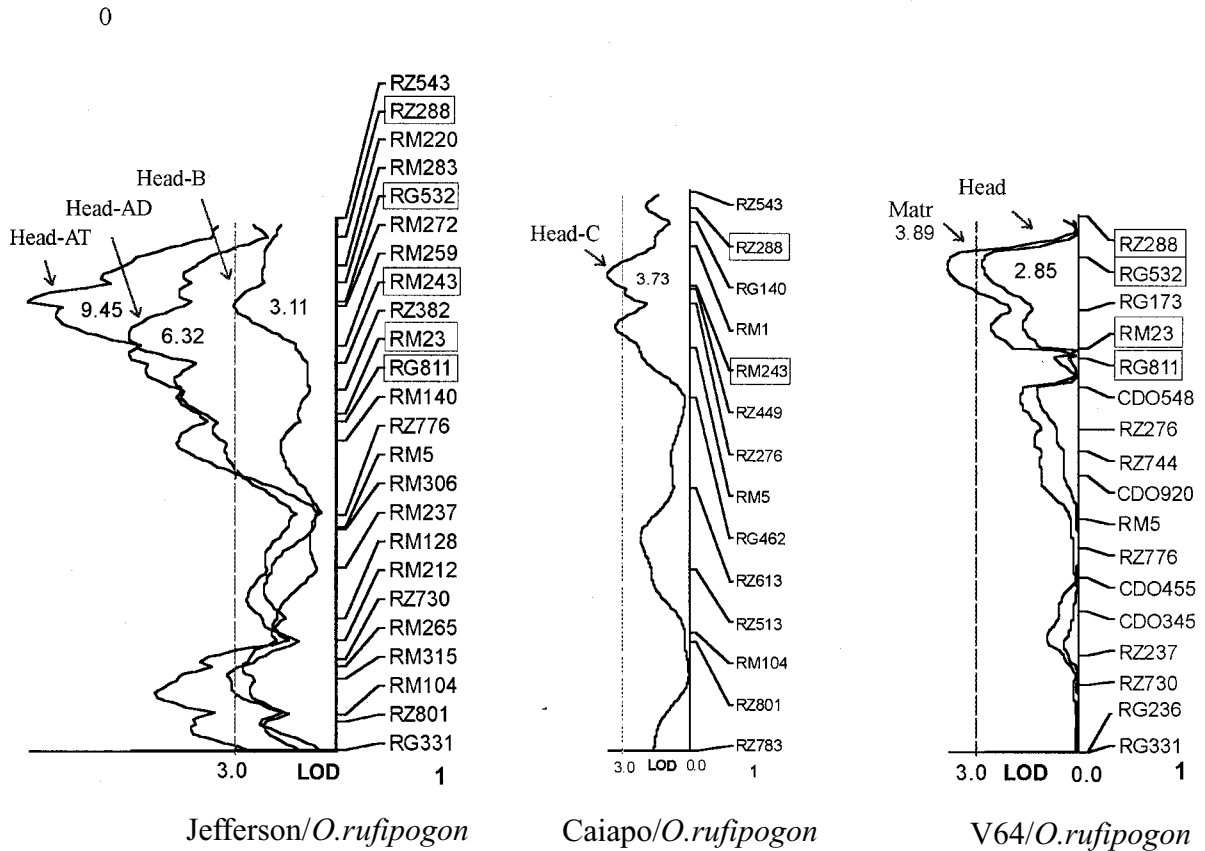


**Fig. 1.** Near-isogenic line development for the *yld1.1* QTL on chromosome 1: NILs are being developed by marker-assisted selection on V20B and three restorer lines: Ce64, MH63, and Wan3 (Jiming Li, personal communication). Two examples of SSCP patterns are given for markers RM129 and RM5.



**Fig. 2.** Comparison of yield QTLs on chromosome 1 in the region of RM5. A yield QTL from an RI population of Milyang23 and Gihobyeo was identified in this region (Cho et al., 1998; and personal communication). Two yield QTLs were identified from the wild rice variety *O. rufipogon*, one with Milyang23 as the recurrent parent (S. N. Ahn personal communication) and the other in the V64 population (Xiao et al., 1998). A yield QTL from *O. barthii* was also identified in this region (J. Tohme and C. Martinez, personal communication).





**Fig. 3.** Comparison of interval analysis plots of heading date QTL on chromosome 1. The *O. rufipogon* allele contributes to earliness in the Jefferson and Caiapo populations, while the V/64 allele contributes to earliness in the V/64 population. The three lines in the Jefferson plot represent three environments: Head-AT is Alvin, Texas (transplanted plants; peak LOD score = 9.45), Head-AD is Alvin, Texas (drilled plants; LOD 6.32), and Head-B is Beaumont, Texas (LOD 3.11). The Caiapo data is from Columbia (LOD 3.73); while the two lines in the V64 plot represent heading date and maturity for a single field environment (peak LODs = 2.85 and 3.89, respectively). Markers flanking the QTL that are shared between populations are outlined.

**Table 1. Advanced Wild QTL Projects.**

Population	Structure	Families	Field data	Markers	QTL analysis
V20/Ce64/ <i>O. rufipogon</i> <sup>a</sup>	BC2 testcross families	300	1994	122	Completed
V20/Ce64/ <i>O. glaberrima</i> <sup>b</sup>	BC2 testcross families	308	1995	130	Completed
Caiapo/ <i>O. rufipogon</i> <sup>c</sup>	BC2 families	274	1996	125	Completed
Jefferson/ <i>O. rufipogon</i> <sup>d</sup>	BC2 families	353/258	1998	156	In progress
IR64/ <i>O. rufipogon</i> <sup>e</sup>	BC2 families	286	1998	84+	In progress

<sup>a</sup> Xiao et al., 1998. Marker data and QTL analysis at Cornell, field data in China.

<sup>b</sup> Li et al., unpublished. Marker data and QTL analysis at Cornell, field data in China.

<sup>c</sup> Moncada et al, unpublished. Marker data and QTL analysis at Cornell, field data in Columbia and Brazil.

<sup>d</sup> Tai and Thomson et al., unpublished. Marker data and QTL analysis at Cornell, field data in Texas and Arkansas.

<sup>e</sup> Septiningsih et al., unpublished. Marker data and QTL analysis at Cornell, field data in Indonesia.

## THE INTERNATIONAL RICE GENOME PROJECT

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### ABSTRACT

Progress in agricultural and environmental technologies is hampered by a slower rate of gene discovery in plants than animals. The vast pool of genes in plants, however, will be an important resource for insertion, via biotechnological procedures, into an array of plants, generating unique germplasms not achievable by conventional breeding. To accelerate the discovery of plant genes, many scientists around the world have participated in building an infrastructure of physical and genetic maps from few plants, including a number of crop species. However, DNA sequence information from these species is still very small compared to animal and microbial organisms. Since the task of generating substantial DNA sequence information from species with good resolution maps can be very costly, a more selective approach has to be taken. While single-pass cDNA sequencing from a dozen species would be very rapid and cost-effective, whole genome sequencing would be more comprehensive and accurate, but it is limited because of the higher cost. Since plant genomes vary so much in size, whole-genome sequencing calls for example of widely studied plants with a small genome. Among the crop species, the rice genome stands out because it is a true diploid genome with only 400 MB, about three times the size of the Arabidopsis genome that has one of the smallest plant genomes. Still, after the human genome, rice has the largest one that would be targeted for complete sequencing. Because of the size of such a project, plans have emerged during the summer and the fall of 1997 to formulate the framework for laboratories from different countries to participate. This framework was finalized Tsukuba, Japan, on 5 February 1998 by the rice genome working group representing China, Europe, Japan, Korea, and the United States. Because 20,000 cDNA sequences from *Oryza sativa* ssp. japonica cultivar. Nipponbare, also known as GA<sub>3</sub>, have been determined and a physical map based on YACs that cover more than 50% of the genome has been published, GA<sub>3</sub> has been selected as the sole DNA source. PAC/BAC libraries are now being constructed in Japan and the United States to generate genomic clones for sequencing. In the early phase, emphasis will be placed on sequencing the ends of PAC/BAC clones with universal primers. For instance, a 60,000 member BAC library with an average insert size of 130 kb should yield 120,000 ends. With an average read of 350 nucleotides the BAC-end sequence database alone would already contain more than 10% of genome size. Establishing such a searchable database in the early phase will simplify and accelerate the international coordination. Once a member of the above library has been mapped to a chromosomal starting position and sequenced in its entirety, its sequence can be compared to the BAC-end sequence database to identify another member of that library with minimal length of overlap. Sequencing can be continued in BAC increments without new physical mapping and resequencing large regions of previous clones, starting from one defined chromosomal location. Because the BAC libraries and endsequence database are publicly accessible, sequencing centers all over the world can proceed limited only by their own resources. However, to ensure coordination and to avoid duplication of effort, meaningful participation will require a minimal yearly commitment of 1Mb and clearing chromosomal starting positions by the rice genome working group. Furthermore, all participating groups will have to adhere to certain data quality and release policies.

### VISION AND GOALS

Fundamental plant biological information from a model plant: As a member of the Graminae and a crop plant, a wealth of fundamental information about important aspects of plant biology can be learned from the genomic sequence of rice.

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Rice is a model for learning about yield, hybrid vigor, single and multigenic disease resistance. Different races of rice are adapted to a wide variety of environmental situations, from tropical flooding to temperate dry land, so it is a model for real life adaptive responses. Because it shares collinear genomes, rice is a key to knowledge of the genomic organization of the other grasses. Comparison of the sequence of the dicot, *Arabidopsos thaliana*, with that of rice, a model monocot, will tell us what genome structures these two different groups of angiosperms have in common and how they differ.

While the goals of the International Rice Genome Project must be focused, the information provided by the International Project can be exploited by the entire community to learn:

- The function and map location of cereal and ultimately all plant genes.
- Use of map-based sequence information to identify and provide markers for agronomically significant genes.
- The molecular basis of plant growth and development so that fundamental questions in plant physiology, biochemistry, cell biology, and pathology can be addressed.
- The relationship, if any, of genome structure to gene expression.

The primary goal is the complete genome sequences of rice.

The primary activity in the first year will be to prepare and distribute clones for sequencing. During this period, it is anticipated that the libraries will be quality controlled and that the clones will be end sequenced and fingerprinted. Subsequent years will be devoted to large-scale genomic sequencing. The objective is to complete the task in ten years.

The time line below for the first five years indicates that greater than 170 MB of the 430 MB genome will be sequenced by 2003, that chromosomes 6 and 10 will have been completed, and the sequencing of chromosomes 1 and 2 will be well underway. The purpose of the international collaboration is to accelerate the completion of this goal.

The International Collaboration is best achieved by sharing materials and technologies and by the timely release of sequences and related information. To this end, scientists interested in the genome sequencing of rice participated in a workshop held in conjunction with the International Symposium on Plant Molecular Biology in Singapore on September 23, 1997 (<ftp://genome.bio.bnl.gov/pub/maize/rice.html>). A Working Group, nominated in Singapore, met on 5 February 1998 to develop this document.

### **Membership in the Rice Genome Project**

Any group willing to sequence large stretches of contiguous DNA is welcome to join the collaborative effort as long as they are willing to follow the agreed upon guidelines. Participants agree to share materials, including libraries, and to the timely release to public databases of physical mapping information and annotated DNA sequences. A group must agree to sequence one megabase of DNA per year to maintain membership. Members agree to declare their sequencing plans and to provide detailed plans and progress on their respective web pages.

Individual sequencing groups are encouraged to claim large chromosomal regions or entire chromosomes, if they have the sequencing capacity, to increase the likelihood that entire chromosomes are completed. Groups may claim chromosomal regions which they agree to sequence within one to three years.

Post-sequencing activities, such as functional genomics, are beyond the scope of the International Rice Sequencing Project. Further, the Project does not encompass the cloning and sequencing of specific rice genes for research purposes or industrial sequencing efforts. While the International Project will be happy to share information with these individual efforts, their conduct is beyond the scope of these agreements.

### **The Rice Genome Working Group**

The Working Group is the body that will make decisions that pertain to the goals, strategies, and coordination of the collaborative effort. The Working Group will be responsible for planning the most efficient means of completing the project. Among its responsibilities will be assigning regions to be sequenced that will avoid duplication and maximize overall progress.

The Working Group is comprised of representatives of each research group participating in the International Rice Genome Sequencing Project. As Japan is recognized as having a leadership role in the Project, the head of the RGP will be the permanent chairman of the Working Group.

Major policy decisions, including sequencing assignments, will be taken by representatives from each of the major national groups participating in the Project. Currently, these regional representatives are Japan, China, Korea, Europe and the United States.

The Working Group will meet annually in Japan. Interim meetings, as needed, may be held elsewhere. The meetings will be open to the public. Results of Working Group meetings will be posted on web sites and published in the RICE GENOME.

## METHODOLOGY

The *Oryza sativa* ssp. japonica cultivar Nipponbare, also known as GA<sub>3</sub>, will be sequenced. Seed from a single plant will be distributed by Dr. Sasaki for the purpose of making libraries. The primary reasons for choosing this cultivar are that more than 20,000 EST sequences from the strain have been released to DDBJ and that a physical map based on YACs that covers over 50% of the genome has been published. Sequencing other cultivars is strongly discouraged as genetic polymorphisms cannot be distinguished from sequencing errors. Moreover, groups not sequencing from one of the shared libraries would not benefit from the associated accumulated knowledge and the other advantages of collaboration. It is recognized that comparative mapping and sequencing of other rice subspecies is valuable information that the International Rice Genome Sequencing Project would like to share. Nevertheless, the primary goal of the Project is the complete sequence of the genome of a single cultivar.

The RGP will make a PAC library each with a 20-fold genome coverage. Dr. Rod Wing will make three BAC libraries using partial digests of different enzymes to generate the inserts. 60,000 BAC clones will be isolated to provide a 20-fold coverage of the genome. The quality of these libraries and their coverage will be verified by hybridizing each with 100 single copy EST probes and the number of clones and their insert size will be measured. It is expected that inserts will be greater than 120 kb. The number of clones with organellar DNA and rRNA repeats will also be determined.

In parallel with fingerprinting, the BAC and PAC clones will be subjected to end-sequencing. This should provide an STS every 3 to 4 Kb on average and will allow genome sequencers to pick the clones with minimum overlap.

### Accuracy

The Rice Genome Sequencing Project will serve as a model for all other grasses and cost about \$200M. The sequence will be used by other researchers and will thus be scrutinized. It is imperative that these resources not be squandered on inaccurate results. In part, this problem has been addressed by insisting on sequencing DNA from the same cultivar, if not the same plant, to minimize variation due to genetic polymorphism.

Fingerprinting of multiply overlapping inserts is a means of verifying that the BACs chosen for sequencing have not been rearranged. Collinearity with the genome should also be verified by probing restriction enzyme digests of genomic or the appropriate YAC DNA with the BAC and comparing this with digests of the BAC itself.

The Rice Genome Sequencing Project will adopt the standards of The Human Genome Project, established at its Bermuda meetings in 1996 and 1997, which has agreed to accept a standard of less than one error in 10,000 bp. While the level of accuracy is difficult to verify, this standard is achievable by a combination of high quality shotgun sequence reads, a seven-fold redundancy, and the insistence 97% of all bases be sequenced on both strands or two chemistries used. In addition, minimum error estimation values provided by PHRED of 75 over protein coding regions and 40 over the remainder of the genome must be obtained. Further, restriction sites predicted from the sequence must conform to observed digest patterns.

### Sequence Release

The Rice Genome Sequencing Project agrees to the immediate release of finished, but not necessarily annotated, sequence in units of intact BAC or PAC inserts. The finished sequences will confirm the accuracy standards described above. Release is submission to a public database such as DDBJ, EMBO, or GenBank. In keeping with the NHGRI recommendations, automated release of assemblies greater than 2 Kb to local Web sites is encouraged.

### Annotation

Members of the Working Group, while recognizing the importance of annotation to the value of sequence information, view annotation as separate from release of finished sequence. Each sequencing group is responsible for annotating the sequence they contribute. A uniform standard of annotation has been agreed upon that checks the integrity of the sequence, assigns and identifies regions of homologies, delineates potential open reading frames, and names and indicates the beginnings and ends of genes. Common annotation software will be adopted.

The annotator must state whether coding sequences and splice sites were determined experimentally or by using software. It is recognized that the use of published cDNA sequences greatly facilitates this task. If gaps cannot be closed, the method of sizing and the reasons for not closing must be stated.

Exact details on how adjacent BACs or PACs were assembled with minimum overlap of 100 bp should also be stated.

It is hoped that annotation will be expanded to include recognition of genetic markers, ESTs, known genes, and syntenic regions. An annotation workshop is projected for the Working Group meetings.

### **Rice Genome Database**

An integrated database established in Japan will facilitate collaboration, coordinate sequencing work, and provide methods for submitting, using, and sharing information. Sequences will be release to one of the public databases, DDBJ, EMBO, or GenBank. The Rice Genome Database will pick up new submissions from the public databases. The Databases will store and manage the annotation information. Each participant will maintain a Web site with a standardized format that describes work in progress and sequences completed. The Database will be linked with the Web sites of each of the Projects participating laboratories and thus be able to maintain a registry of clones being sequenced, monitor progress, and coordinate activities. The database will also be linked with sites that are providing finger printing information and end sequences. With ever expanding databases, annotation is never complete. It may be advisable to assign the task of periodic update of the annotation of rice genomic sequence to the Rice Genome Database.

The larger goals for the Project envision the use of sequence information to provide biological lessons for rice and other cereals. The Rice Genome Database is a means for linking all genomic information related to rice DNA sequence. This information comes from existing genomic databases and from work that derives from DNA sequencing, such as determination of gene function. The Rice Genome Database will be linked with other rice and cereal databases and to international groups that will be learning about the function of rice and other cereal genes.

### **Outreach**

To be successful, this large sequencing effort needs the broad support of scientists working on rice and other cereals who will be the potential end users of the sequence information. Ultimately, it is the public at large who supports the project and steps at public education should be undertaken. They must believe that the project is worthwhile, that is well-organized and credible. There are a number of ways that The Rice Genome Sequencing Project will attempt to engender this support:

- Timely release of finished, annotated sequence blocks, as well as the availability of mapped BACs and YACs.
- RICE GENOME will report the results from the Working Group meetings as well as news of the Project.
- Internet access to The Rice Genome Database will engender awareness and use of the Project.
- Publications from participating sequencing laboratories should acknowledge that they are part of the Project.

**POSTERS  
(ABSTRACTS)**

## **GENETIC DIVERSITY OF KOREAN-BRED RICE CULTIVARS FROM 1933 TO 1998**

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### **ABSTRACT**

Although there is much concern about genetic vulnerability, little is known about the recent changes in the genetic diversity of rice in Korea. The objective of this study was to know the trends in the genetic diversity of Korean-bred rice cultivars. The genetic diversity of 167 rice cultivars developed by three breeding stations (NCES, NHAES, and NYAES) from 1933 to 1998 was assessed using 27 RAPD markers polymorphic at 96 loci and 10 microsatellite markers representing 10 loci. 124 cultivars are japonica and the remaining 43 are Tongil type. Among the 124 japonica rices, 12 are anther culture-derived varieties. NCES developed 72 cultivars, 50 were by NHAES, 39 were by NYAES, 3 by selection from traditional varieties, and 3 are foreign introductions. A total of 150 RAPD alleles was resolved in all the cultivars. Some alleles occur in one of the two groups (japonica vs. tongil) with a high frequency. The level of allelic diversity of the materials was compared by ecotype and breeding stations. Also diversity was compared across Korea at 10-year intervals. The results will provide an understanding of the trends in the genetic diversity of Korean-bred cultivars and their use in future breeding work.



## **A FAST METHOD FOR RICE BREEDING USING INDUCED MUTATIONS**

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### **ABSTRACT**

Induced mutations for disease resistance in cereals were considered promising fields in the last decades. In attempts to develop more improved varieties of rice, gamma irradiation has been used to induce mutations. In this work, seeds of the commercial variety Cr 1821 were irradiated with gamma rays from a cobalt 60 source, using a dose of 250 Gy. The  $M_1$  plants raised after mutagenic treatment were segregated according to the spike progeny method, and under *Pyricularia* inoculation mutant selection was performed by repeated quantitative assessment of the disease symptoms under field conditions in selected and propagated. Each of the mutant lines was evaluated for disease resistance, lodging resistance, yield and grain quality. From them, one of the mutant lines was selected for its performance against disease, higher nitrogen response, good milling quality, and 20% higher yield than the parent variety. The line was called CAMAGO 8 and was officially released three years and four months after the initial seed irradiation. The results showed that mutation breeding could be a fast and practical method for rice improvement.

## IMPROVING BASMATI RICE THROUGH INDUCED MUTATION

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### ABSTRACT

Basmati rice has a great importance for Pakistan. Because of its high quality and flavor, it fetches premium price in the domestic as well as international markets and is one of the main sources of foreign exchange earnings for the country. The prevalent basmati varieties are tall growing, take longer to mature, are not fertilizer responsive, are susceptible to lodging and are not high yielding like the IRRI varieties. Since the efforts to evolve higher-yielding varieties of basmati by conventional cross breeding always seemed to result in progenies having lesser or no basmati aroma it was felt that inducing mutations may succeed because the basmati background will be retained. Our efforts at inducing genetic variability in basmati background resulted in the evolution of short-statured and shorter duration mutants. Further selection and trials resulted in the release of a shorter duration variety, "Kashmir Basmati," now well established in Azad Kashmir and other northern areas.

Some short-statured, higher-yielding mutants are also in advanced stages of evaluation. An advanced mutant DM-25 gave much higher yield (4.5 tons/ha) than Basmati 370 (2.9 tons/ha) and was at par with the most prevalent, though less aromatic variety Basmati 385 (4.58 tons/ha). DM-25 also showed resistance against diseases and is now under consideration of the Punjab Government for release as a commercial variety. Genetic studies of the induced dwarf mutants revealed that the dwarfing gene in DM 107-4 is non-allelic to the DGWG source. The mutant has been registered in the international gene pool and has been allotted the gene symbol  $d^{59}$ . This is perhaps the only dwarfing source in the basmati background, and can be used in the breeding programs aimed at the improvement of basmati rice.

## PHYLOGENETIC STUDY OF AA GENOME WILD RICE SPECIES BY PHENOTYPE AND ISOZYME ANALYSIS

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### ABSTRACT

Variability of 21 quantitative characters and 29 isozyme loci were studied for five AA genome wild species; *Oryza rufipogon*, *O. glumaepatula*, *O. meridionalis*, *O. longistaminata*, and *O. barthii*. Polymorphisms at phenotype and isozyme loci were respectively examined by principal component analysis and scatter diagrams were generated using the first and second principal component scores.

*O. rufipogon* and *O. glumaepatula*, showing higher phenotype variability than *O. meridionalis* and *O. barthii*, were divided into two and three clusters in the scatter diagram, respectively; *O. rufipogon*-I, II and *O. glumaepatula*-I to III. *O. rufipogon*-I, and *O. glumaepatula*-I, which were categorized as perennial type, were plotted overlapping each other. While, *O. rufipogon*-II joined *O. meridionalis* and *O. barthii*, forming an annual type group. On the other hand, isozyme analysis showed that five species formed respective clusters without overlapping, indicating that they are genetically differentiated from each other. Phenotypes are subjected to natural selection. The plants, even with different phylogenetic backgrounds, are likely to develop similar phenotypes when they grow under similar environments. The above mentioned perennial wild rices prefer stable and less disturbed environments where density-dependent mortality is frequent, while annual types tend to inhabit unstable and occasionally disturbed environments where density-independent mortality is frequent. Judging from the incongruence between inter- and intra-species variation pattern revealed by phenotype and genotype, similarities found in phenotypes are most probably homoplacy caused by evolutionary convergence.

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# INCIDENCE OF DISCOLORED KERNELS IN SELECTED JAPANESE, KOREAN, AND AUSTRALIAN RICE CULTIVARS GROWN IN ARKANSAS

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## ABSTRACT

Rice kernels can be discolored by rice stink bugs, diseases such as kernel smut and brown spot, and by a physiological response possible to high temperatures. Whatever the cause of kernel discoloration, rice that has high amounts of discolored kernels often will receive price discounts by rice buyers. Avoiding high amounts of discolored kernels can be achieved by insect management or selection of rice varieties less susceptible to insect damage, disease infection, and damage by high temperatures. Evaluations of rice lines for susceptibility to discolored kernels are important information for rice breeding programs when rice lines from other countries are evaluated for desirable traits. This report is a summary of evaluations of selected rice lines from Japan, Korea, and Australia for amounts of discolored kernels.

The number and origin of rice lines, and the years evaluated were: (1) 1993 and 1994, 56 Japanese lines found in the USDA germplasm collection and 4 check varieties; (2) 1995, 40 Japanese, 12 Korean, and 3 Australian lines recently placed in the germplasm collection and 11 check varieties; and (3) 1996, the 40 Japanese lines evaluated in 1995, 37 of the Japanese lines evaluated in 1993 and 1994 with heading dates of 90 days or less, and 12 check varieties. Rice was hulled with a McGill #2 sheller and discolored kernels were manually separated from other kernels. Discolored kernels were then examined with magnification and categorized by the cause of the discoloration. The amount in each category was expressed as a percentage of the total brown rice weight.

The categories of causes of discolored kernels were (1) rice stink bug, (2) kernel smut, (3) linear damage - a straight (linear) disruption on the kernel surrounded by a dark brown or black area thought to be a reaction to high temperatures, and (4) other types of discolorations including kernels with discolored bran. All Japanese, Korean, and Australian varieties had very few kernels discolored by kernel smut. In 1993 and 1994, the long-season (>90 days to heading) Japanese short- and medium-grain varieties had more discolored kernels due to rice stink bug than did the earlier heading Japanese varieties. For the same years linear damage was much higher in Japanese and check varieties from California than that found in the check variety Mars. Japanese varieties grown only in 1995 and 1996 had nearly the same moderate amounts of discolored kernels due to rice stink bug and high amounts of linear damage. When grown in 1996 the 40 Japanese varieties also grown in 1993 and 1994 had higher amounts of rice stink bug damage and linear damage. Koshihikari had high levels of rice stink bug damage (>3%) and moderate levels of linear damage (>1%). Akitacamachi had high levels of rice stink bug damage (>2%) and very high levels of linear damage (>4%).

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## **GERMPLASM WITH GENETIC MALE-STERILITY FOR RICE RECURRENT SELECTION BREEDING**

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### **ABSTRACT**

The collaborative Rice Project between the Centre de coopération internationale en recherche agronomique pour le développement (CIRAD - France) and the Centro Internacional de Agricultura Tropical (CIAT- Colombia) emphasizes population breeding using recurrent selection. The Project aims at: (1) developing, for different Latin American Rice Ecosystems, broad base gene pools and populations segregating for a male-sterile gene, (2) enhancing the germplasm using recurrent selection (3) developing, from the enhanced populations, fixed lines and potential donors through conventional breeding, and (4) strengthening national rice programs. Six populations introduced from Brazil (former collaborative project between CIRAD and EMBRAPA) and from French Guyana (CIRAD), were evaluated in Colombia. Four populations were locally developed by introduction of new variability into the better-adapted populations. For the tropical lowland ecosystem of Latin America, three populations (PCT6, PCT-7, and PCT-8) were developed. They are being evaluated and enhanced in Argentina, Colombia, Costa Rica, El Salvador, Panama, and Venezuela. For the temperate lowlands ecosystem, two populations (GPIRAT-10, and PQUI-1) currently are being evaluated and enhanced in Latin America (Argentina, Brazil, Chile, and Cuba), and Europe (France, Hungary, and Romania).

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## SELECTION OF SALT-TOLERANT BASMATI RICE VIA *IN VITRO* TECHNIQUES COUPLED WITH IRRADIATION<sup>1</sup>

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### ABSTRACT

Rice is one of the most important cereal crops of Pakistan. However, the average yield of rice in Pakistan is the lowest among the major rice-producing countries. One of the main constraints for the low yield of rice in Pakistan particularly Basmati is that it is mainly grown on soils having moderate salinity with high pH. Generally rice yield from salt affected soil is about half as compared to that from normal soil. In Pakistan, about 5.7 million hectares of land has been classified as saline or saline sodic. It therefore seems logical to develop basmati rice varieties/germplasm having resistance against salinity through modern breeding techniques.

Keeping in view these objectives, somaclonal mutants are being developed from Basmati rice CV. Basmati 370 through combined use of tissue culture and gamma irradiation. Culturing of embryogenic calli into modified MS regeneration medium supplemented with 25 mg/l ABA slowed down the growth of embryogenic calli but improved their conversion rate to plants in hormone free MSo medium. Kinctin and NAA promoted the growth of somatic embryos but failed to improve conversion rate. Combined use of ABA and PGRs (K + NAA) could not trigger plant regeneration. Among first somaclonal generation (SC1) only four plants at 0.2% of NaCl and one at 0.4% of NaCl under gravel culture were screened. The progeny of these screened mutants yielded two salt-tolerant mutants with 17% and 33% sterility. The growth value of adapted callus lines at 0.2%, 0.25%, and 3.0% of NaCl *in vitro* was less than unadapted callus lines. These lines are under investigation *in vitro* for regeneration potential.

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<sup>1</sup> This research work is partly financed by IAEA under research contract no.7645/RB.

**LARGE-SCALE SCREENING OF USDA RICE GERMPLASM  
FOR QUALITATIVE AND QUANTITATIVE RESISTANCE TO  
TWO COMMON RACES (IC17 AND IB49) OF THE RICE  
BLAST PATHOGEN *Pyricularia grisea***

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Over 700 entries in the USDA germplasm collection were screened for both qualitative and quantitative resistance to the rice blast pathogen, *Pyricularia grisea*. The germplasm was screened with each of two races of the pathogen, IC17 and IB49, in a large-scale inoculation format whereby 400 entries could be screened per inoculation. Races IC17 and IB49 are common races which predominate throughout Arkansas. Qualitative resistance was evaluated based on disease reactions after one infection period (approximately seven days) whereas quantitative resistance was evaluated after multiple inoculations (one, two, three, and four weeks). The purpose of these efforts were to begin developing a database of resistance genes in the USDA germplasm collection based on bioassays to races which represent concerns to rice production in the state. Identification of germplasm with qualitative or quantitative resistance genes can then be used to combine different qualitative resistance genes as well as qualitative and quantitative resistance genes in an effort to develop rice cultivars with improved durable resistance to rice blast. A wide range of qualitative and quantitative resistance responses were observed among the diverse collection of germplasm.

## WILD RELATIVES: A POSSIBLE SOURCE OF RESISTANCE TO RICE DISEASES

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Wild relatives often have served as additional sources of disease resistance genes for crop plants. Sheath blight (*Rhizoctonia solani* Kühn) and blast (*Pyricularia grisea* Cav.) are the major fungal diseases affecting rice (*Oryza sativa* L.). The objective of this study was to introduce additional sources of sheath blight resistance from wild relatives of rice (*Oryza* species) into southern U.S. rice cultivars. *O. rufipogon* (PI 590420) and 27 IRRI accessions reported as sheath blight resistant and representing eight wild *Oryza* species, were crossed with the long-grain experimental line, RU9401188 and the medium-grain cultivar, Bengal. F<sub>1</sub> progeny were backcrossed to the cultivated rice parent. Embryo rescue is being used in attempts to obtain hybrids not acquired by emasculation and crossing. Pollen stainability and self seed set were determined for the *Oryza* species, F<sub>1</sub> and BC<sub>1</sub> plants. Ratoon plants of *Oryza* species and F<sub>1</sub> hybrids were inoculated with *R. solani* and rated on a 0 to 9 scale for resistance. To date, F<sub>1</sub> progeny were obtained from crosses with 21 of the 28 accessions. All but one of the successful crosses was with an A genome species, *O. barthii*, *O. glumaepatula*, *O. meridionalis*, *O. nivara* or *O. rufipogon*. One C genome, *O. officinalis* accession (IRRI no. 101399) hybridized with Bengal and most likely with RU9401188. Backcross (BC<sub>1</sub>) progeny were derived from crosses with 17 accessions and BC<sub>2</sub> progeny from crosses with 12 accessions. Also, the *Oryza* species are being rated (0 to 9 scale) for resistance to the five races of blast commonly found in the United States.



**NATURAL SUPPRESSION OF BARNYARDGRASS  
(*Echinochloa crus-galli*) AND RED RICE (*Oryza sativa*) BY  
SELECTED RICE CULTIVARS**

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Weeds often reduce yield and quality of rice in the southern United States. Among the more economically important weeds, barnyardgrass (*Echinochloa crus-galli*) has developed resistance to herbicides in some cases, and red rice (*Oryza sativa*) cannot be controlled adequately in rice by current weed control practices. Recent research at Stuttgart has indicated that some aggressive rice cultivars may be capable of suppressing establishment and growth of some of the important weeds of rice in the South, including barnyardgrass and red rice. A series of field experiments were conducted at Stuttgart from 1995 to 1998 to evaluate and better understand the natural weed suppression potential of rice germplasm lines and commercial cultivars to barnyardgrass and red rice in dry seeded rice systems. Several foreign rice lines, including PI 312777 from the Philippines and Teqing from China, have frequently maintained high grain yields and low barnyardgrass biomass compared to commercial U.S. long-grain standards when treated with propanil at rates as low as one-half to one-fourth of normal use rates. Delaying initial flush irrigations or rainfall for more than one week after rice planting appears to enhance the overall suppression of barnyardgrass, especially in the foreign rice lines. In more recent studies, PI 312777 has moderately suppressed the establishment and growth of red rice. Collectively, these results suggest that future rice production may benefit both agronomically and economically from growing aggressive and/or allelopathic rice cultivars that aid in cultural control of the major weeds of rice.

## **RICE GERmplasm EVALUATION AND ENHANCEMENT AT FLAR**

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The Fund for Latin American and Caribbean Irrigated Rice (FLAR) was created in 1995 by organizations from the public and private sectors to maintain international research efforts. FLAR members are Brazil, Colombia, Venezuela, Cuba, Costa Rica, Panama, Guatemala, Paraguay, Uruguay, CIAT, IRRI, and CIRAD (France). The basic research objectives are to continue the access and interchange of germplasm and information via INGER-LAC, identify and characterize new progenitors for use in the region's breeding programs, develop new cultivars with stable resistance to major biological and edaphic stresses in the region, investigate post-harvest aspects of the crop, and promote strategies of crop management which lead to a more sustainable production. As a service to member countries, FLAR maintains a rice quality laboratory; evaluates breeding lines for blast and Hoja Blanca disease, iron toxicity and cold tolerance, and makes crosses on demand. In 1998 we evaluated over 7600 F2 families from 338 crosses, 1174 F4 populations from 19 crosses, and 620 R2 lines from 58 crosses. We also screened 650 introductions from different LAC countries, CIAT, IRRI, and CIRAD. Since the inception of FLAR we have made over 954 triple crosses and have characterized a working germplasm collection of over 470 entries. The selected lines from our trials are distributed to FLAR members in early generations (F5), or through INGER-LAC. For 1999 we expect to send over 100 well characterized breeding lines to our member countries, as well as 150 lines to participate in INGER-LAC. This work is important to the region to insure the continued availability of diverse and adapted germplasm to researchers and producers.

## RETROSPECT OF RICE IMPROVEMENT AND BREEDING STRATEGIES OF SUPER HIGH-YIELDING RICE VARIETIES IN CHINA

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Rice is the main staple food in China. A brief history of rice improvement in China was outlined and discussed in four phases: 1) Evaluation and utilization of traditional tall-stature varieties (From 1949 to 1960); 2) The utilization of semi-dwarf genes and development of semi-dwarf varieties (From 1956 to present); 3) Research and development of hybrid rice (From 1964 to present); 4) The breeding of new cultivars and combinations of hybrid rice with higher yield, multiple-resistance, good grain quality (From 1990 to present).

“Semi-dwarf breeding” and “Hybrid rice breeding” are two milestones in rice improvement. Since the late 1980s, rice yield has reached a high plateau. The unit yield increase rate of the first five-year period in 1990s (0.2 %) was much lower than that of 1970s (2.14%) and 1980s (4.07%). If this trend is not reversed, severe food shortages will occur in the country at the turn of the century. A special collaborative research project on breeding super high-yielding rice was initiated by the Ministry of Agriculture of China in 1990. Some examples of super high yield varieties are presented with yield potential of 11.25t/ha, which is a 10 to 15% increase over the best currently available varieties.

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<sup>1</sup>Hu Peisong, visiting scholar at Texas Agricultural Experiment Station, Beaumont.

## **A NEWLY DEVELOPED LATE-SENESCENCE MUTANT IN RICE**

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A late-senescence mutant was newly induced by the treatment of N-methyl-N-nitrosourea on fertilized egg cells of a Korean japonica rice variety, Hwacheongchalbyeo. The mutant kept green leaves even after accomplishment of grain filling due to less degradation of chlorophyll in leaves, while the original variety turned yellow around 25 to 35 days after heading. The reduction rate of leaf chlorophyll content showed similar response to several fertilizer applications even though the absolute chlorophyll content differed along treatments. Chlorophyll a/b ratio was not changed in the mutant. Under cold temperature, the mutant still maintained green leaves and showed a better growth than the original one, indicating that the mutant could be used as a cold-tolerant germplasm. The grain filling pattern of the mutant was the same as the original variety. The photosynthetic rate of the mutant during the ripening stage, however, seemed to be different from the original variety. Study on the association of grain filling with photosynthetic rate in this mutant is going on. The morphological and grain characteristics of the mutant was the same as the original variety under ordinary cultivation practices. Fingerprinting both the mutant and the original variety using 85 microsatellite DNA markers showed 100% genetic similarity between them, confirming that the mutant should be induced by a point mutation. The inheritance mode of the late-senescence trait of the mutant was examined using several F1 and F2 populations from the crosses between the mutant and varieties. The result indicated that late-senescence trait was controlled by a single recessive gene. This mutant is expected to be useful for breeding cold-tolerant and submergent -tolerant varieties. The nature of late-senescence, its agronomic usefulness, and gene tagging work is in progress.

## **GENETIC IMPROVEMENT OF RICE IN AUSTRALIA - A KEY FACTOR IN SUSTAINABLE RICE PRODUCTION**

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Rice production in the temperate region of southern Australia now exceeds 1 million tons of paddy annually. The industry is based largely on japonica germplasm, although a suite of varieties have been developed which satisfy consumer demands in most of the major rice quality categories. Around 85% of production is exported.

Water availability is a key issue facing the industry, and is a result of seasonal variation in supply and of competing demands from the wider community. Low minimum temperatures during critical growth stages are a major contributor to rice yield variability, and management of water depth has proved a useful tool for ameliorating temperature damage. Solving the puzzle of tolerance to low temperatures is therefore a key issue, and would allow freedom to examine a much wider range of strategies to improve water use efficiency. This, in turn, would minimize the impact on the environment.

The recently formed CRC for Sustainable Rice Production will boost the research effort. The main areas being addressed include tolerance to cold during seedling establishment, early pollen microspore development and flowering. Agronomic, physiological and genetic studies are in progress to understand mechanisms, which can be used to breed for resistance to cold. Greater cold tolerance will feed directly into the wider research effort to improve the sustainability of rice production in Australia.

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## PROGRESS IN SEARCH OF APOMIXIS IN RICE

*Y.C. Li<sup>1</sup> and J. N. Rutger<sup>2</sup>*

Pre-developed embryo rice lines (PDER) were obtained by ion implantation. Embryological observations confirmed that PDER possessed diplospory at a frequency of 5 to 10%. 1) Some embryo sacs with two nuclei next to micropyle end indicated some megaspore mother cell (MMC) which developed directly into diploid embryo sac with eight nuclei without meiosis process. 2) Elongated and enlarged ovaries on panicle four days after strict emasculation and isolation were found to have embryo and endosperm, but have no pollen on their stigmas. It indicated that diploid egg and polar nuclei divided spontaneously into embryo and endosperm without pollination and fertilization. However, apomixis is strongly connected to polyploidy. In the expectation that polyploidy might enhance the expression level of apomixis, PDER and Ce-64 were treated with colchicine, resulting in successful induction of tetraploid PDER and Ce-64. Crosses were made among 4N PDER, 4N Ce-64, 4N L-202, and 4N Jackson. Tetraploid hybrid F<sub>1</sub>'s had higher rates of seed set than their tetraploid parents, and some approached seed set levels of diploids. Fluorescence observations demonstrated that there are weak fluorescence reaction of 51 to 70% MMC in 4N hybrid F<sub>1</sub> like in *E. Rectisetus* with diplospory which demonstrated that there may be higher diplospory in 4N hybrid F<sub>1</sub>.

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## THE ROLE OF SEED INFESTED BY *Pyricularia grisea* IN THE EPIDEMIOLOGY OF RICE BLAST

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Field and greenhouse experiments were conducted in 1996 and 1997 to determine if seed infested with *Pyricularia grisea* could serve as a primary inoculum source. Artificially infested seed using a sulfate non-utilizing (sul) mutant of *P. grisea* was applied to the soil surface at plant emergence at rates of 0, 0.5, 5, 25, and 50 seed per 0.93 m<sup>2</sup> in plots planted with the susceptible cultivar 'M201.' Leaf blast symptoms were first detected in the inoculated plots after 45 days in both 1996 and 1997. The results indicate that the onset of disease was more dependent on the number of days after planting than on plant maturity. Blast was not detected in the control plots (no infested seed) in 1997 and not until 65 days after plant emergence in 1996. Disease increased in all treatments in 1996, with the most disease in the highest treatment (50 infested seed per 0.93 m<sup>2</sup>). However, even in the lowest treatment (0.5 infested seed / 0.93 m<sup>2</sup>), leaf blast incidence was greater than 40% by the end of the season. In 1997, low levels of leaf blast were detected in the 5, 25, and 50 infested seed per 0.93 m<sup>2</sup> treatments, while blast was not detected in the lowest treatment. At the end of the season, the sul mutant was recovered from more than 90% of the leaf, collar, and neck blast lesions indicating inoculum from infested seed applied at the beginning of the season initiated and perpetuated an epidemic. The incidence of *P. grisea* infecting or infesting rice seed grown in Arkansas was determined by microscopic examination following incubation on moistened filter paper. Seeds were collected randomly from different growers planting foundation, registered and non-registered seed. The incidence of *P. grisea* identified on rice seed incubated on moistened filter paper ranged from 0 to 10%. Incidence was generally greater on the most susceptible cultivars sampled.

## **GENES FROM WILD RICE CAN CONTRIBUTE TO YIELD INCREASE IN CULTIVATED RICE**

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The *Oryza* wild species represent a potential source of new alleles for improving yield, quality, and stress resistance of cultivated rice. We have initiated the implementation of a marker assisted breeding program using the Advanced Back-cross QTL method that will lead to the development of improved cultivars. Two improved rice cultivars (Bg90-2 and Lemont) were crossed to *O.rufipogon* and *O.barthii*, respectively. 300 BC2F2 (Bg90-2/*O.rufipogon*) and 326 BC3F2 (Lemont/*O.barthii*) families were developed and evaluated for main agronomic traits, including grain yield under irrigated conditions at CIAT, Palmira. Transgressive segregation for grain yield was detected in the Bg90-2/*O.rufipogon* cross, with several BC2F2 families having between 5 and 25% higher yield than Bg90-2. The results were confirmed from replicated trials of BC2F3 families. Molecular analysis, using 90 RFLPs and 14 microsatellites from the RF-Cornell map, indicated positive association between yield and QTLs located on chromosomes 2, 3, 5, and 12. In the Lemont/*O.barthii* cross, data from 326 BC3F2 showed that several families yielded up to 30% more than Lemont. Molecular analysis is underway to detect positive QTLs associated with yield increase in this cross. Results suggest that both *O.rufipogon* and *O.barthii* have genes that can contribute to yield increase in cultivated rice.



## UTILIZATION OF *Oryza rufipogon* TO IMPROVE CULTIVATED U.S. RICE

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Recent research has shown promise in using wild species to improve cultivated crops for yield and quality traits (Tanksley, et al., 1996 and Xiao, et al., 1996). This study was initiated to evaluate the potential of using the wild species *Oryza rufipogon* to improve a southern U.S. long grain rice cultivar (*Oryza sativa*), Jefferson. The *O. rufipogon* donor parent (IRGC 105491) is characterized as being tall, pubescent, awned, high tillering, late maturing, dormant, susceptible to shattering and having a black hull, medium grain shape, and an apparent amylose content of 22 to 24%. In contrast, Jefferson, is a semi-dwarf cultivar that is glabrous, awnless, low tillering, early maturing, not dormant, resistant to shattering, and has a straw colored hull, long grain shape, and an apparent amylose content of 20 to 22%. Both Jefferson and *O. rufipogon* were observed to be resistant to races IC17 and IE1k of the blast fungus, *Pyricularia grisea*. Divergent reactions were observed between the two parents for reaction to race IB49 (Jefferson is susceptible) and race IG1 (*O. rufipogon* is susceptible) of blast. Three hundred fifty-three backcross progeny (BC2F1) were produced at CIAT with the next generation produced in quarantine facilities in Beaumont. Phenotypic measurements were made on the BC2F1 plants while in quarantine and were used to cull out families carrying noxious weed traits (e.g. dormancy and high shattering). A total of 258 BC2F2 progeny were used for QTL analysis and were planted in a replicated field trial in 1998. A summary of the QTL analysis will be presented using data collected under greenhouse conditions. These data will be compared with field data that are being collected in the 1998 field trial.

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## **RICE GERMPLASM EVALUATION AND UTILIZATION IN INDONESIA**

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Significant contribution of rice breeding to agricultural development, especially in rice production, has indicated the important role of high-yielding varieties developed. Through the use of landraces as well as introduced germplasm, in the first long-term development 96 high-yielding varieties have been released. Currently, 85% of the total rice land has been planted with some of those released varieties. Varietal release has been done in a gradual manner. The important characters of the released varieties in one period reflected the response to the need in that particular period, which was indicated by the number of varieties vastly grown in certain period. Among the agricultural technologies developed, high-yielding variety has the significant role in increasing yield per unit area as well as the primary component of integrated pest management. Our past experience has taught us that planting large areas with single variety resulted in genetic vulnerability caused by the outbreak of insect pests and diseases. For example, the outbreak of brown plant hopper in 1970s caused almost total yield loss. This indicated the important of breeding program for specific location. Therefore, rice germplasm evaluation is not only for resistance to insect pests and diseases and environmental stresses but also for adaptation to different rice growing ecosystems.

## **PHENOTYPIC CHARACTERIZATION OF A RICE POPULATION, GP IRAT 10, AND ITS USE IN RECURRENT SELECTION**

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The use of the recurrent selection method (RS) in rice breeding is relatively new, and its importance has increased after the availability of a recessive gene for male-sterility, found as a mutant in the cultivar IR36. The inclusion of that gene in a rice population, enables the use of RS, significantly increasing the crossing rate among individuals. The population GP-IRAT 10, synthesized by CIRAD-CA, has a wide genetic background of japonica genotypes, and includes the male-sterility gene from IR36. The purpose of the work was the analysis of this population to check its possible adaptation to local temperate climate, and to determine its potential use for developing breeding lines and for implementation of a RS program. A group of 3000 plants was transplanted during the 1995-96 growing season at INIA Treinta y Tres. A random sample of 300 marked plants was used to evaluate 26 traits, including some continuous and discontinuous variables, and several statistical parameters were used in the analysis. The population showed, in the average, non desirable plant architecture, due to open tillering and non erected leaves, as well as excessive plant height and long growth duration. It also showed some desirable traits, as high tillering ability with high effective tillering, and good panicle size. Grain sterility was moderate, indicating an acceptable adaptation to local climate. The obtained results show that GP IRAT-10 has low value for direct use, and that it will be necessary to apply high selection pressure in order to get desirable genotypes. That management, however, may result in narrowing the genetic base of the population. The strategy for implementation of RS, since 1996/97 growing season, has been to apply high selection pressure and to include new variability into the gene pool, through new parents with desirable agronomic traits, synthesizing new populations and maintaining the original male-sterility gene.

## INHERITANCE OF SHEATH BLIGHT RESISTANCE AND DEVELOPMENT OF RESISTANT RICE LINES THROUGH RECURRENT SELECTION

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A modified recurrent selection program was initiated in 1992 to develop rice breeding lines with multiple sources of partial sheath blight (SB) resistance, good agronomic characteristics, and high yield potential for use in the Rice Research Station breeding program at Louisiana State University. All known resistance to SB is partial. Sources of partial resistance included were LSBR-5, LSBR-33, Teqing, Jasmine 85, Gui Chow, H<sub>4</sub>/CODF, LB86-30344, Rice/Grass, Leah, Katy, Tetep, Taducan, CICA 6, CICA 9, Yangdao 4, Rax Clear, and *Orzica llanos* #5. The inheritance of partial SB resistance was examined in crosses between the resistant genotypes H<sub>4</sub>/CODF, LB86-30344, Jasmine 85, Teqing, Gui Chow, and Yangdao 4 and the susceptible varieties Lemont, Maybelle, and Cypress. Parents, F<sub>1</sub>, F<sub>2</sub>, BC<sub>1</sub>F<sub>1</sub>, F<sub>3</sub>, F<sub>4</sub>, and F<sub>2-4</sub> progeny plants were evaluated for disease resistance in the field from 1994 to 1997. The results indicated that partial SB resistance from these sources was a dominant character controlled by single dominant genes. A single dominant gene, designated *Rh2*, was found in H<sub>4</sub>/CODF, LB86-30344, and Jasmine 85. The single dominant gene found in Gui Chow and Teqing, designated *Rh3*, segregates independently from *Rh2* and has additive effects when combined with *Rh2*. Plants from F<sub>2</sub> populations for crosses between Yangdao 4 and Lemont segregated in a 9 resistant:7 susceptible ratio, suggesting complimentary dominant gene control of the partial SB resistance. These resistance sources were crossed with susceptible commercial varieties and among themselves. Partially resistant progeny from these crosses were crossed to give progeny with multiple resistance genes. This process is on-going, with 768 crosses made to date. Approximately 13,000 progeny rows are evaluated annually in field plots inoculated with the SB pathogen, *Rhizoctonia solani*, at the Rice Research Station in Crowley, Louisiana, and select rows are advanced to the breeding program at the F<sub>4</sub> to F<sub>6</sub> generations.

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## **RECURRENT SELECTION FOR RICE (*Oryza sativa*) BLAST (*Magnaporthe grisea*) RESISTANCE IN POPULATIONS WITH NARROW GENETIC BASE**

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Recurrent selection is used to enhance populations with large genetic base (PLB) for polygenic traits like partial blast resistance. If the selection index is too high there is a risk of losing useful genes not still in favorable genetic associations. The selection index has to be moderate, so recurrent selection breeding using PLB is a long-term process. The use of recurrent selection in populations with narrow genetic base (PNB) is a mean to avoid the risk of loss of useful genes and speed-up the genetic progress at medium-term.

To develop PNB the first step is to select few parents with the highest known level of expression of a specific trait, for example IRAT 13 for partial blast resistance. At the end of the recurrent selection process the fixed lines would present each trait at the corresponding parent level, for example the partial resistance level of IRAT 13.

Advantages. In comparison with the use of F<sub>2</sub> populations the potential genetic progress is better because the recurrent cycles permit the use of the best parent for each individual trait, without warring for its other qualities. In comparison with the use of PLB the genes are less diluted so the use of PNB requires less time for the development of good lines. The risk of genetic derive is also reduced, and it is possible to fix the PNB for oligogenic traits before the starting of the recurrent selection breeding. The live of PNB is short, so it is easier make new populations than adapt old ones to follow objective changes.

Disadvantages. In comparison with the use of F<sub>2</sub> populations more time is needed to develop fixed lines because the first step is to enhance the PNB before the pedigree selection. The recurrent selection method is not adapted to the breeding for oligogenic traits. In comparison with the use of PLB the potential genetic progress is reduce.

The use of PNB is a technical compromise between the use of F<sub>2</sub> populations and PLB use. The three types of population are complementary parts of a same general breeding scheme.

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## ADVANCES IN DEVELOPMENT AND USE OF HYBRID RICE TECHNOLOGY IN THE TROPICS

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Hybrid rice has contributed significantly to increased rice production in China during the past two decades. However, in other countries adoption of this technology has been limited due to 1) unadaptability and/or unacceptability of the Chinese hybrids and parental lines, 2) restricted availability of the Chinese germplasm, 3) lack of confidence in the economic viability of the technology and/or, 4) inadequate human resources and infrastructure to develop and use the technology. During the past two decades IRRI has been engaged in research, training, and consultancy services to overcome these constraints and has made following contributions to help the interested NARS to develop and use this technology.

- Confirmed yield advantage (at least 1 t/ha) of rice hybrids, derived from parental lines adapted to the tropics, over inbred grown under irrigated ecosystem in the tropics and sub-tropics.
- Developed suitable parental (viz., A, B, and R) lines for sharing with public and private institutions working in NARS to develop heterotic rice hybrids.
- Shared continuously elite IRRI experimental hybrids with NARS for evaluation and use.
- Developed hybrid rice seed production technology which can give up to 3 t/ha seed yields and packaged it in the form of manual video movie and slide tape module for training purposes.
- Recognized the fact that specific agronomic management strategies are needed for maximizing yield expression of hybrids; these strategies are somewhat different from those used for inbred.
- Recognized that grain quality of hybrids was dependent on the grain quality of their parental lines thus making it possible to develop hybrids with desired grain quality characteristics.
- Recognized the fact that resistance of hybrids to biotic stresses was associated with resistance of parental lines and nature (dominant, recessive) of resistance gene(s) thus, making it possible to develop hybrids with desired resistance to diseases and insects by using appropriate parental lines.
- Developed some thermosensitive genic male sterile lines to breed two line hybrids.
- Developed some CMS, maintainer and restorer lines possessing wide compatibility gene(s) to breed indica/tropical japonica hybrids possessing enhanced heterosis.
- Developed some CMS and restorer lines possessing basmati grain quality to breed basmati rice hybrids.
- Found that hybrid rice technology was economically viable in India, Vietnam and Philippines if the hybrids showed at least 1 t/ha yield advantage over inbred and if seed yields of at least 1.5 t/ha was obtained.
- Trained more than 150 rice researchers and seed production personnel through degree, non-degree and on-the-job training.
- Provided consultancy services to several NARS directly or in collaboration with FAO for establishing and/or strengthening their hybrid rice programs.

Because of the above stated developments, 18 countries outside China have initiated hybrid rice research and seed production programs in collaboration with IRRI. Several private seed companies in India, Philippines, Indonesia, and Vietnam have invested in hybrid rice research and/or seed production and marketing. India, Vietnam, and the Philippines have commercialized hybrid rices in 120,000, 180,000 and 100 ha, respectively in 1997. Other countries are still developing the technology and hope to commercialize it within the next five years by which time India, Vietnam, and the Philippines plan to cover 2 million, 0.8 million and 50,000 ha, respectively under hybrid rices. IRRI, FAO, and several NARS have established an International Task Force on Hybrid Rice (INTAFOHR) to expedite the development and use of hybrid rice technology. To strengthen these efforts: IRRI in collaboration with FAO and Asia Pacific Seed Association (APSA) and six countries (viz., Bangladesh, India, Indonesia, Philippines, Sri Lanka, and Vietnam) have initiated a project on "Development and Use of Hybrid Rice in Asia," which aims to expedite development and use of hybrid rice technology.

## **RICE GERmplasm COLLECTION AND MANAGEMENT SYSTEM IN THE UNITED STATES**

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A total of 17,279 rice accessions including 10 species of *Oryza* are currently in the USDA-ARS rice germplasm working collection. The accessions have been collected from 110 countries or regions. *Oryza sativa* is the major species in the collection with 17,044 accessions from 109 countries or regions. The International Rice Research Institute (IRRI) has supplied 1,025 accessions from the Philippines, 2,280 IR breeding lines and 2,378 accessions from other countries, and is the largest group from one location in the collection. The second largest group is from China (1,793), and the third largest group is from India (1,269). The U.S. rice germplasm collection includes land races, varieties, advanced breeding lines, and genetic stocks. Currently, 31 descriptors are listed in the germplasm resources information network (GRIN) for rice. GRIN is the centralized computer database system that manages plant germplasm documentation and movement throughout the national plant germplasm system (NPGS). NPGS is a network of organizations and people dedicated to preserving the genetic diversity of plant germplasm. Members of NPGS include Federal, State, and private organizations. The Agricultural Research Service (ARS) coordinates the system. GRIN can be accessed through the Internet (<http://www.ars-grin.gov>) or through pcGRIN which is a personal computer version of GRIN that includes data about one or more crops to accommodate the PC and floppy diskette. Through GRIN national and international scientists can locate germplasm with specific characteristics for research purposes.

## EXPLORATION OF RICE GERMPLASM IN CENTRAL VIETNAM IN 1997

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The exploration of rice germplasm in the central Vietnam was carried out in November, 1997. This was the sixth mission in a cooperative project of Japan and Vietnam for rice germplasm collection since 1994.

In 1997, we visited the provinces in central region, Quan Nam, Kon Tum, Gia Lai, Dac Lac and Lam Dong. The villages we visited in Quan Nam was at the altitude of 200m from the sea level along rivers. The other four provinces were in the central highland. Provinces of Kon Tum, Gia Lai and Dac Lac were at the altitude of 500 to 700m and Lam Dong, 1,000 to 1,500m. The central highland was the area of relatively dry weather and of red soil of low fertility. In these area, upland rice was cultivated by slash-and-burn practice besides paddy rice in small paddy field.

In this exploration, 119 local cultivars were collected, among which 98 cultivars were upland cultivars. Preliminary observation of brown rice grain indicated that 22 cultivars were glutinous and 23 cultivars might be low-amylose cultivars, which must be analyzed further.

In the cooperative project of Japan and Vietnam since 1964, 964 local cultivars and one wild relative have been collected. These cultivars and variety have been cultivated in research institutes both in Japan and Vietnam and evaluated for their agronomic traits.

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## CONCLUSIONS

There was open exchange of scientific information amongst participants, which is beneficial to all concerned.

The Symposium covered the full spectrum of international rice germplasm research, from collection, evaluation, enhancement, to rice genome research.

## RECOMMENDATIONS

That rice researchers everywhere make every effort to remain committed to free and open international exchange of scientific information and germplasm.

Efforts should be continued to evaluate existing resources and fill gaps in world rice collection.

The Rice Genome Project is a flagship research project, and all groups are urged to contribute to and/or support this effort.

Rice research efforts should focus on developing environmentally friendly and sustainable technologies for increased food production, at the same time.

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