

Contributions of Molecular Biology to Breeding and Issues Associated with Its Application in Developing Countries

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Abstract

Biotechnology and its scientific base, molecular biology, which encompass a range of scientific applications involving living organisms, could provide the added boost necessary to help agricultural researchers accelerate progress in feeding hungry people in developing countries. Four areas are discussed:

1) the role of biotechnology and molecular biology in developing countries; 2) some accomplishments and basic strategies in molecular biology, particularly for rice; 3) bio- and food safety; and 4) intellectual property (IP). An historical perspective covers accomplishments at IRRI in tissue culture, marker-aided selection, characterizing biodiversity in genebanks, genetic engineering, and structural genomics. The next challenge is in the area of functional genomics, that is, to discover the functions of genes across the genomes of rice and other crops. To enhance efforts in gene discovery in rice, the creation of a public, well-characterized mutant bank through international collaboration is proposed. Regarding bio- and food safety, IRRI and other international centers are committed to contributing good science that will help regulatory bodies make well-informed decisions on the use of genetically modified organisms. Concerning IP, IRRI and other centers must promote the developing world's right to free access to all the resources and tools of the new biotechnology sciences. IP will be a particularly relevant issue for those involved in functional genomics because of the high likelihood that many new products will result from this research.

Introduction

Biotechnology—a term that encompasses a range of scientific applications involving living organisms and that includes the realm of molecular biology, its scientific base — certainly has been on the minds of consumers, farmers, and researchers over the last few years.

Proponents and opponents are all making their views known in newspapers and magazines and on television and the Internet. New terms such as *genetic engineering*, *gene food*, *Frankenfoods*, and *golden rice* have entered the lexicon on one side of the ledger or the other. Sometimes it can all be very confusing. But at the same time, it is also very exciting because of the potential of what the new techniques of molecular biology can bring to our agricultural research efforts to feed hungry people in developing countries and bring stability to the world.

Investments in agricultural research will cultivate peace

Jimmy Carter, the former U.S. President and chairman of the Carter Center, pointed out the importance of agricultural research to world stability when he stated in a recent editorial that “there can be no peace until

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people have enough to eat and investments in agricultural research today can cultivate peace tomorrow” (Carter, 1999). And he went on to say that “responsible biotechnology is not the enemy; starvation is.” Indeed, biotechnology could potentially provide that added boost to agricultural research that will help us accelerate progress in feeding the hungry at a very opportune time in human history.

IRRI’s role: objective evaluation

However, we must underline the word *potential*. IRRI’s role is not to merely promote molecular biology and other biotechnology techniques or the resulting genetically modified organisms (GMOs). Our role is to objectively evaluate the new strategies and options that biotechnology may offer the rice industry and to work with our partners in the national agricultural research systems of rice-producing nations to see if such strategies are suitable and sustainable in different countries — particularly developing countries.

The poor: the greatest beneficiaries of biotechnology

If and when properly tested and evaluated GM rice takes its turn in the paddy fields of Asia, poor rice farmers and consumers will be the greatest beneficiaries. The new varieties will increase production and lower prices. Knowing what benefits would be reaped by the poor, Per Pinstrup Andersen, director general of the International Food Policy Research Institute (IFPRI), stated in a recent op-ed piece in the *International Herald Tribune* that “the developing world simply can’t afford to do without agricultural biotechnology” (Pinstrup-Andersen, 1999). He fears that the heated debate over biotechnology risks closing off a huge opportunity to improve the lives of the small-scale farmers of Asia, Africa, and Latin America.

Echoing Dr. Pinstrup-Andersen’s position this past July were the Academies of Science of Brazil, China, India, Mexico, and the U.S., and the Royal Academy of Science in London and the Third World Academy of Sciences, which jointly urged the increased development and use of biotechnology to improve crops and to solve problems of hunger and poverty in developing nations (Yoon, 2000). Describing genetic engineering as an important tool that can provide more nutritious crops and disease-resistant plants, the seven academies called for increased support from governments and industry, including increased research financing for scientists and freer use of patented gene technologies by farmers in the developing world.

A Gene Revolution

Indeed, all these so-called “gene” technologies have led to the rise of a Gene Revolution — a term coined by Dr. M.S. Swaminathan, a former director general of IRRI and the first World Food Prize Laureate, who was on the cutting edge of an earlier revolution — the Green Revolution. Now at the dawn of the 21st century, Dr. Swaminathan envisions that this new revolution will provide a molecular understanding of the genetic basis of living organisms, as well as the ability to use this understanding to develop new processes and products for agriculture, the environment, and human and animal health (Swaminathan, 1999).

Accomplishments and basic strategies

So, what have been the accomplishments so far of this new Gene Revolution and what basic strategies should we be formulating to keep the spark alive for future generations? Although this paper looks mostly from the IRRI viewpoint with rice as the focus, much can be extrapolated to other crops as well. First, let’s

try to get an historical perspective.

Historical perspective

Before 1960, rice breeders were frequently limited in what they could achieve because of a lack of access to diverse parental lines with the needed characters. But with the founding of IRRI in 1960, the national and international exchange of germplasm has given breeders access to much of what they need. The International Rice Genebank at IRRI houses the world's largest collection of rice germplasm (more than 107,000 samples). These materials are freely available and have greatly increased the scope of rice breeding. The collection contains many wild *Oryza species* other than the Asian cultivated rice, *Oryza sativa*. Some "pre-molecular" tissue culture techniques, such as embryo rescue and anther culture, have made it easier to tap into the biodiversity present in the genebank.

Embryo rescue

Since the mid-1980s, embryo rescue has allowed the recovery of fertile offspring from wide crosses that would otherwise be sterile. By overcoming a sterility barrier, embryo rescue has enabled IRRI breeders to widen the scope of their work by introducing genes from wild species of rice. Using embryo rescue, we have been able to produce hybrids between elite breeding lines or varieties and several accessions of 11 wild species representing eight genomes. Through this technique, IRRI has transferred from wild species useful genes for resistance to brown planthopper, bacterial blight, and blast (Khush and Brar, 1998). Additionally, national program researchers in the Philippines, China, India, Vietnam, and Indonesia are using embryo rescue to produce interspecific hybrids in rice for genome analysis, to determine species relationships, and to derive desirable alien introgression lines.

Anther culture

Anther culture has enabled us to rapidly stabilize new lines in one season rather than the six to eight seasons normally required in conventional breeding. At IRRI, the anther culture technique is being employed to obtain doubled haploid (DH) lines from many traditional and wide crosses for different rice breeding objectives (Khush and Brar, 1998). On average, about 20 to 50 DH lines can be produced from one cross and to date IRRI has regenerated more than 8,000 DH lines. We are working to increase the efficiency to 100 lines per cross in indica rice (Alejar *et al.*, 1995). Breeders have selected several superior DH lines with one being released as a salt-tolerant variety in the Philippines. In addition to the Philippines, research institutions in China, Korea, India, Vietnam, and Indonesia are using anther culture in their plant breeding programs to produce elite homozygous breeding lines as well as mapping populations.

Marker-aided selection

By 1988, we had moved into the molecular biology arena with DNA-based techniques that allowed us to accelerate traditional breeding with DNA marker-aided selection (MAS). MAS, which enhances selection efficiency for agronomic traits, has delivered some of the promises of biotechnology, at least in rice (Tanksley and McCouch, 1997).

Table 1 shows 11 genes for blast resistance, eight genes for bacterial blight resistance, and 16 genes for various other useful traits that have been tagged with molecular markers located on the 12 chromosomes of rice (Khush and Brar, 1998). How do we use this information? In Asia, for example, four resistance genes (*Xa4*, *xa5*, *xa13*, and *Xa21*) for the devastating bacterial blight have been introduced in various combinations into locally adapted varieties. The IRRI-based Asian Rice Biotechnology Network (ARBN) is facilitating the

sharing of these elite lines and gene pyramids across many Asian political boundaries. In the Punjab of India, the popular variety PR106, carrying two-gene and three-gene combinations, has been produced and is being evaluated by farmers. In Indonesia and the Philippines, single- and two-gene pyramids for bacterial blight resistance have been introduced into the popular IR64 variety (Persley and Lantin, 2000).

Thanks to MAS, we can now more efficiently breed for bacterial blight resistance by accumulating multiple resistance genes and we can be more efficient in incorporating salinity tolerance by minimizing environmental “noise.” In the very near future, we will be breeding for drought tolerance during the wet season.

In a new effort, we are extending the application of MAS to search for diverse traits in the gene pool. IRRI researchers are collaborating in molecular breeding with scientists from 30 research and plant breeding institutions in 15 countries to introgress different genetic characteristics of 60 elite rice varieties currently grown on more than 60% of Asia’s rice lands. Another 143 exotic varieties known to have important traits to contribute to genetic improvement are also being used (IRRI, 2000b).

Characterizing biodiversity in genebanks

To assist crop breeders, genebank curators have been using DNA markers (such as random amplified polymorphic DNA [RAPDs], amplification fragment length polymorphisms [AFLPs], and recently more robust microsatellites) to exploit genetic resources — including untapped wild species — in their collections in a way that they never could with traditional morphological and agronomic evaluation.

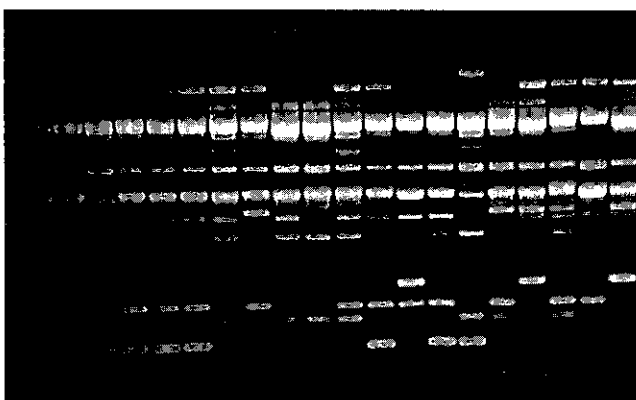


Fig. 1 RAPD profile of 26 accessions of *Oryza barthii*, an AA genome wild species from sub-Saharan Africa, generated by primer OPR19

Table 1 Thirty-five agronomically important genes mapped with rice chromosome identified

Gene	Trait	Rice chromosome
<i>Pi1</i>	Blast resistance	11
<i>Pi2</i>	“	6
<i>Pi4</i>	“	12
<i>Pita</i>	“	12
<i>Pi5</i>	“	4
<i>Pi6</i>	“	12
<i>Pi7</i>	“	11
<i>Pi9</i>	“	6
<i>Pi10</i>	“	5
<i>Pi11</i>	“	8
<i>Pib</i>	“	2
<i>Xa1</i>	Bacterial blight resistance	4
<i>Xa2</i>	“	4
<i>Xa3</i>	“	11
<i>Xa4</i>	“	11
<i>xa5</i>	“	5
<i>Xa10</i>	“	11
<i>xa13</i>	“	8
<i>Xa21</i>	“	11
<i>RTSV</i>	Rice tungro spherical virus resistance	4
<i>Bph1</i>	Brown planthopper resistance	12
<i>Bph10</i>	“	12
<i>ef</i>	Early flowering	10
<i>fgr</i>	Fragrance	8
<i>Gm2</i>	Gall midge resistance	4
<i>Rf3</i>	Fertility restorer	1
<i>S5</i>	Wide compatibility	6
<i>Se1</i>	Photoperiod sensitivity	6
<i>Se3</i>	“	6
<i>sdg</i>	Semidwarf	5
<i>sd1</i>	“	1
<i>tms3</i>	Thermosensitive male sterility	6
<i>PMS 1</i>	Photoperiod-sensitive male sterility	7
<i>PMS 2</i>	“	3
<i>Sub1</i>	Submergence tolerance	9

Source: Khush and Brar (1998).

The exploitation of cross-species similarities through comparative genetics offers great potential to identify traits in rice germplasm using data from other cereal crops (Bennett, 2000).

In rice, IRRI is using several marker systems to understand the diversity of wild species, such as the RAPD profile in Fig. 1 of 26 accessions of *Oryza barthii*, an AA wild species from sub-Saharan Africa. Information here will help us manage the material in the genebank and provide some insights into how it might be strategically used in breeding programs. We have also used molecular markers to identify duplicate accessions and to predict the performance of rice accessions in the field (Jackson, 1999). The advantage of using both RAPD and AFLP markers is their broad distribution across the rice genome, based on data from a wide diversity of rice varieties (Jackson, 1999).

We have one word of caution, especially for genebank curators in developing countries with relatively low budgets. Too often, there is pressure to begin work with molecular biology for its own sake. However, we believe that it is essential that the basic elements of a strong conservation program be in place first before developing biotechnology capabilities in a genebank. Otherwise, the added benefits that biotechnology can bring may not be realized, or germplasm may not be readily available on which to apply the advanced techniques if it has not received proper care in the genebank (Jackson, 1999).

Genetic engineering

By combining the tried-and-true applications of tissue culture with the new and exciting applications of molecular biology under the genetic engineering umbrella, scientists can now transform the rice crop by moving the genes within rice around very selectively or moving useful genes from other species directly into rice.

Protoplast transformation began at IRRI in 1991 — after successes in Japan with japonica rice (Shimamoto *et al.*, 1989) and in Switzerland with indica rice (Datta *et al.*, 1990). By 1993, we were doing microprojectile bombardment and, in 1996, we started *Agrobacterium*-mediated transformations. Several elite rice cultivars, which were transformed with single and hybrid *Bacillus thuringiensis* (*Bt*) genes, have shown 100% insect resistance (Datta *et al.*, 1998), and deepwater rice with built-in plant protection can now be grown in the fragile ecosystems of poor farmers (Alam *et al.*, 1998).

We now have transgenic IR72 with the *Xa21* gene (from the wild species *Oryza longistaminata*) and a transgenic commercial hybrid rice, “Shan You 63,” with a *Bt* gene. In field evaluations in China, both of these biotechnological creations have shown excellent resistance to bacterial blight and stem borers, respectively (Tu *et al.*, 2000a,b). In addition, the transformation that made news worldwide in January was the insertion in Switzerland of three genes (two from daffodil and one from a bacterium) into the japonica variety T309 to create the beta-carotene-enriched golden rice (Ye *et al.*, 2000).

IRRI is currently collaborating with Japanese institutions in genetic engineering, including Tokyo University in lodging tolerance and iron enrichment, Meiji Seika Kaisha, Ltd., in fungal resistance, and Nagoya University in transferring the C_4 photosynthetic pathway to rice. IRRI is also looking forward to future collaboration with JIRCAS in developing rice with a regulatory gene conferring tolerances to cold, salt, and drought.

Use of these techniques by developing country researchers

Several developing countries are starting to produce tangible outputs derived through the application of molecular biology techniques. For example, researchers at the Research Institute for Food Crops Biotechnology (RIFCB) in Indonesia have incorporated MAS products—the *xa5* and *Xa7* bacterial blight (BB) resistance genes — into IR64. The Indonesian group, as part of the ARBN, is conducting thorough field evaluations to confirm the presence of the BB genes by using molecular markers. RIFCB plans to release two cultivars — Bio-1 and

Bio-2 — that represent some of the first MAS products generated by developing-country teams associated with the ARBN.

In addition to the Indonesian team, other developing-country teams have set up impressive laboratory protocols and work plans. Each ARBN country team has developed a work plan that guides the conduct of its research activities (Table 2). India is particularly active with projects going on in four institutions where they are using MAS to enhance several important traits. China, the Philippines, Thailand, and Vietnam are also using molecular techniques to enhance rice varieties.

The country plans are periodically refined or modified based on mutual agreement between the team and IRRI. The ARBN teams continue their collaboration with IRRI, particularly through workshops held either in the IRRI laboratories or in-country.

Molecular biology priorities at IRRI

IRRI researchers are currently enhancing 34 traits in five key areas (Table 3) for rice through molecular biology approaches — by either MAS or genetic engineering or both. For MAS, some genes — such as for tungro, blast, and bacterial blight resistance, phosphorus deficiency, and yield components — have been transferred into *Oryza sativa* through wide hybridization. Some traits — such as for drought tolerance and yield — are also being improved through the International Molecular Breeding Program. And some complex

Table 2 Work plan emphasis of developing countries in the ARBN using molecular biology applications

China

- China National Rice Research Institute (CNRRI)— Molecular characterization of bacterial blight and blast pathogen populations of India and breeding for cultivars with bacterial blight and blast resistance genes
- Guangdong Academy of Agricultural Sciences (GAAS)— Application of molecular methods to enhance gall midge resistance in rice and to understand gall midge populations in South China

India

- Central Rice Research Institute (CRRI)— Molecular characterization of bacterial blight and blast pathogen populations of India and breeding for cultivars with bacterial blight and blast resistance genes
- Directorate of Rice Research (DRR), India—Marker-aided selection for development of rice varieties with a broad range of gall midge resistance through gene pyramiding
- Indira Gandhi Agricultural University (IGAU)— Application of molecular methods to enhance gall midge resistance in rice
- Punjab Agricultural University (PAU)— Application of molecular markers to characterize *Xanthomonas oryzae* pv. *oryzae* and deploy bacterial blight resistance genes in variety PRI06

Indonesia

- Research Institute for Food Crops Biotechnology (RIFCB)— Population genetics of important rice pathogens and crop improvement in Indonesia

Philippines

- Philippine Rice Research Institute (PhilRice)— Using molecular markers in rice variety improvement

Thailand

- Department of Agriculture (DOA)— Developing sustainable Thai rice varieties with integrated molecular marker technologies and breeding

Vietnam

- Agricultural Genetics Institutes (AGI)/National Institute of Plant Protection (NIPP)— Development of blast-resistant and high-yielding rice varieties for deployment in Vietnam
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Source: Asian Rice Biotechnology Network.

traits — including sheath blight and stem borer resistance and drought tolerance — could be enhanced by MAS and/or genetic engineering using genes discovered through plant genomics.

Plant genomics for crop improvement

In recent years, scientists have been learning to use the tools of plant genomics to help make even better use of MAS and genetic engineering by greatly accelerating the process of discovering new genes and the traits they control. It is with genomics — the science of determining DNA sequences and their biological functions — that we are at a true departure point for planning breeding strategies for the 21st century.

Structural genomics: Where are the genes?

Over the last 15 years or so, one of the important developments in plant molecular biology has been the construction of molecular genetic maps-known as structural genomics — for numerous crop species (Khush and Leung, 2000). Various molecular markers such as restriction fragment length polymorphisms [RFLPs], RAPDs, cDNA clones, AFLPs, and microsatellites have been mapped and densely populated maps have been prepared for tomato, maize, wheat, barley, and particularly rice. The molecular map of rice, currently consisting of some 2,300 such markers, is the most densely populated of any of the crop species.

The ultimate detailed molecular map will be the complete sequence of all 12 rice chromosomes. This has been the objective of the International Rice Genome Sequencing Project (IRGSP). Under the leadership of the Rice Genome Research Program (RGP), based in Tsukuba, Japan, this effort has expanded to an impressive consortium of at least 10 countries from rice-growing and non-rice-growing regions, which are steadily sending in new information from across the 12 chromosomes in the rice genome. In addition, in April 2000, Monsanto announced the release of the first “working draft” of the rice genome and that it will provide the data to the IRGSP. This additional large infusion of data will enable the international community to complete the rice genome sooner and at a lower cost than we ever dreamed. With this commitment and collaboration from the public and private sectors, a completely decoded rice genome should soon be publicly available.

The rice molecular genetic map will prove to be of stupendous value in improving the crop and will enhance the efficiency and end results of molecular techniques such as characterizing biodiversity in genebanks, gene pyramiding, tagging genes of economic importance, quantitative trait loci (QTL) analysis, and map-based gene cloning. Scientists will also be able to compare the locations of markers across the maps of other species-particularly in cereal species (such as rice and wheat)-to find genes controlling similar traits (Khush and Leung, 2000).

Functional genomics: what can these genes do for breeders?

But now, we have an even greater challenge than completing the rice sequencing project: discovering the useful functions of all those genes, perhaps some 30,000 when the map is finished! It is through functional genomics that we identify the functions of gene sequences by analyzing when and how and which genes work together to generate a trait. To a large extent, it is analogous to the extensive germplasm screening that has allowed conventional breeding programs to extract useful traits, but with precision at the DNA level (Hossain *et al.*, 2000).

Here is another perhaps easier-to-grasp analogy: a completely sequenced genome is like a dictionary with all the necessary words, but without the meanings assigned to those words. We must complete the dictionary by learning the meaning of the words. Then we must use the words to compose essays, that is, to develop new rice varieties (IRRI, 2000a). A complete dictionary of sequences and functions will take us to the next level in plant breeding.

As sequencing work continues on the rice genome roadmap, the genomics team at IRRI is acquiring

Table 3 Priorities at IRRI for DNA-MAS, genetic engineering, and traditional approaches in enhancing key traits in rice

Traits	Priority			Comments
	1	2	3	
Biotic stress tolerance				
Tungro*	TRAD	MAS	GMR	MAS is under development for genes transferred from <i>Oryza rufipogon</i>
Bacterial blight*	MAS	TRAD	GMR	MAS allows pyramiding of genes of similar effect, focus of functional genomics
Blast*	TRAD	MAS	GMR	MAS allows pyramiding of genes of similar effect, focus of functional genomics
Sheath blight	GMR	MAS		MAS is under development through functional genomics
Stem borer	GMR	MAS		MAS is under development through functional genomics
Brown planthopper*	TRAD	MAS		MAS allows rapid introgression of resistance
Gall midge	MAS	TRAD		MAS allows pyramiding of genes of similar effect
Nematodes*	MAS	GMR		MAS for genes transferred from <i>O. glaberrima</i> and <i>O. longistaminata</i>
Abiotic stress tolerance				
P deficiency*	MAS	TRAD		G x E interactions favor use of MAS
Zn deficiency	TRAD	MAS		MAS is under development
Fe toxicity	MAS	TRAD		G x E interactions favor use of MAS
Al toxicity*	MAS			G x E interactions favor use of MAS
Salinity	MAS	TRAD	GMR	MAS allows pyramiding of QTLs for different mechanisms of tolerance
Drought#	TRAD	MAS	GMR	MAS and GMR are under development through functional genomics
Cold	MAS	TRAD	GMR	G x E interactions favor use of MAS
Elongation ability*	TRAD	MAS		MAS is under development
Submergence	TRAD	MAS	GMR	Large-scale TRAD screening is routine
Hybrid rice				
Fertility restorer (Rf)	MAS	TRAD		MAS saves time and labor
Wide compatibility (Sn5)	TRAD	MAS		MAS saves time and labor
TGMS	MAS	TRAD		MAS saves time and labor
Apomixis	GMR			In absence of natural apomixis in genus <i>Oryza</i> , GMR is only feasible approach
Yield and architecture				
Yield*#	TRAD	GMR		GMR under development for starch biosynthesis
Yield components*#	TRAD	MAS		MAS is under development
Fewer tillers	TRAD	MAS		MAS is under development
Large panicle	TRAD	MAS		MAS is under development
Grain number	TRAD	MAS		MAS is under development
C4 photosynthesis	GMR			In absence of natural C4 rice, GMR is only feasible approach
N transfer to grain	TRAD	MAS		MAS is under development
N2-fixation	GMR			In absence of suitable natural N2-fixation, GMR is only feasible approach
Perennial rice	TRAD	MAS		MAS under development
Grain quality and human nutrition				
Aroma	MAS	TRAD		Major QTL
Zn in grain	TRAD	MAS		MAS is under development
Fe in grain	MAS	TRAD	GMR	G x E interactions favor MAS, GMR is under development
Vitamin A in grain	GMR			Yellow pigment of Amarillo rice endosperm is not yet identified

*Trait for which genes have been introgressed from wild species into *O. sativa* and are being developed for MAS.

#Trait that is a focus of near-isogenic line development through International Molecular Breeding Program.

Notes:

MAS = DNA marker-aided selection (includes use of linked markers, candidate genes or identified genes).

GMR = genetically modified rice.

TRAD = traditional breeding and selection.

information as it becomes available. One technique to discover what important agronomic traits the newly discovered genes might control is to produce large numbers of rice plants called "deletion mutants." These special plants have specific genes deleted. By observing the plants and identifying missing characteristics, laboratories at IRRI and in Japan are assigning functions to the missing genes.

To enhance this particular promising avenue of gene trait discovery, it is proposed that a rice mutant bank be created and then maintained in the public domain. Several laboratories in Japan are the world's leaders in using mutants to discover new genes and IRRI will seek to work closely with them to accelerate trait discoveries in rice.

IRRI believes that functional genomics is so critical to the future of rice research that it will be devoting an entire project within its new 12-project medium-term plan to this area of research. In that project, to commence in January 2001, IRRI will be employing a genome-wide experimental approach that will provide unprecedented power to find new genetic information to allow dissection of the metabolic pathways of the genomic map. IRRI will then be in a strong position to use all the genomic databases available and to promote accessibility of this resource to rice-growing countries, particularly those of the developing world.

Bio-and food safety

As mentioned earlier, all of this exciting science has not made us oblivious to the important issues of biosafety and food safety. Indeed, what are the health and environmental ramifications of creating transgenic rice by, for example, moving insect resistance genes from the *Bt* bacterium into rice to combat stem borers or transferring beta-carotene genes from daffodil into rice to fortify the grains with vitamin A, or adding to our future arsenal the genes currently being discovered by our genomic scientists across the cereal species?

It appears, that with each passing year, the potential risks of GM foods seem less and less. Our own 1996 World Food Prize Laureate, Dr. Gurdev Khush, believes that vocal critics will soon be muted by mounting evidence of the safety of biotechnology products. He says, "GM or transgenic crops have been widely grown for the last five years, yet no harmful effects on human health or the environment have been detected (Lynne, 2000)."

In fact, transformed maize, cotton, and potato varieties carrying the insect resistance *Bt* gene have been huge commercial successes in several countries. In 1999, nearly 12 million hectares of *Bt* crops were grown by farmers in Australia, China, South Africa, Spain, and the USA — with no detected harm to human health or the environment (James, 1999).

Many researchers are very disturbed if there is even a chance that the deployment of the new technologies might be delayed in Asia by the protests of those opposed to GM crops (Normile, 2000). In fact, Dr. Khush advocates the earliest introduction of genetically modified rice to Asia. But even with such enthusiasm from among our own ranks, we must be — and are — cognizant of the concerns of many about the safety of the new techniques.

The United Nations stands somewhere between those who claim that GMOs will be the salvation of humanity and those who warn that they could be the curse of the 21st century. UN Secretary-General Kofi Annan stated in the UN's Millennium Report that "Advances in agricultural biotechnology may help developing countries by creating drought-, salt-, and pest-resistant crop varieties." He added, "But, the environmental impact of biotechnology has yet to be fully evaluated and many questions related to biosafety remain to be answered (Polk, 2000)."

IRRI researchers are looking for factual answers — especially where rice is concerned — to the very questions being raised in the media by the UN, NGOs, and people in developed countries. A case in point involves IRRI's work with *Bt* rice. No transgenic rice varieties of any kind have been released so far to

farmers in Asia. Although IRRI scientists continue to seek the best ways to deploy *Bt* rice to delay the buildup of *Bt* resistance in pest populations, they are also conducting concomitant research, funded by USAID, to predict the possible consequences of the movement of *Bt* or other transgenes from transgenic rice into wild and weedy rice populations. When promising *Bt* or other transgenic rice varieties are ready for extensive field testing and release to farmers, this information will be available to assist regulatory bodies in making well-informed decisions about possible risks in their countries (Cohen *et al.*, 1999). IRRI has a responsibility to contribute good science that will help with this decision-making process.

Societies in Europe, North America, and Japan must have the freedom to debate the pros and cons of the development and consumption of GMOs. But it would be wrong for such debate to impede basic research to study whether such technologies are safe, sustainable, and suitable for rice-producing nations of the developing world. Such countries must be allowed the right to make their own decisions on biotechnology, which they cannot do if access to such technology is denied to them.

Plant variety protection and intellectual property

Institutions such as IRRI must not only advance MAS, genomics, and biotechnology as they relate to agricultural research but must also promote the developing world's right to free access to all the resources and tools of these new sciences.

For example, in February 2000, IRRI hosted a 3-day meeting of senior representatives of the 12-nation Council for Partnership on Rice Research in Asia (CORRA), during which a wide range of plant variety protection (PVP) and intellectual property (IP) issues—including those related to biotechnology—were discussed by international experts from the public and private sectors (Padolina, 2000). We expect IP to be a particularly relevant issue for functional genomics because of the high likelihood that many new products will result.

A common collaborative platform

Functional genomics requires diverse genetic resources and expertise. Because of this, it will require a common platform where information and genetic resources can be broadly shared to accelerate trait discovery (Fischer *et al.*, 2000). The key elements of this collaborative platform are as follows: 1) public access to sequence data, 2) abundant genetic resources for functional assignment (such as the proposed gene mutant bank mentioned earlier), 3) capacity for biological evaluation, and 4) incorporation of genes and traits into varieties.

As long as there is a continued commitment to maintain these elements in the public domain, national and international rice breeding programs should be able to make progress as never before. For example, this is why our rice genome scientists — in addition to their efforts in pure science — are working to create a platform to ensure that the vital and powerful information flowing from the advances of genomics remains available to all.

The recent announcements on the release of rice sequence data and donation of proprietary technologies for the production of vitamin A (or golden) rice are truly welcomed. These are encouraging signs that the private sector is willing to share its products and make them available for use by developing countries.

Material transfer agreements

We are hopeful that genetic resources for functional genomics will be made available to the public and private sectors under material transfer agreements or MTAs. These types of agreements would permit recipients to obtain patents on genes discovered through the use of the material, but would require them to make rights under those patents available at a reasonable royalty for application in commercial markets of the

developing world, and at zero royalty for application in research and non-commercial subsistence farming (Fischer *et al.*, 2000).

In addition to ensuring the use of materials and information in the developing world, it will also be essential that these are freely available for research. So, the MTAs would need to have provisions permitting the free use for research purposes of any patents as well as ensuring that patent recipients cannot obtain any form of IP on the genetic stocks.

In short, we are advocating — in rice at least — unique arrangements for specific rights in new inventions derived from biological assets held by the public sector. In addition to public access to information on gene functions in rice and public access to research on any proprietary product derived from the biological assets, we are seeking access for limited use of the products in developing countries.

Conclusion

It is clear that the potential of the new agricultural technologies is tremendous, particularly for improving the lot of poor people in developing countries. Much has already been accomplished with molecular biology techniques such as MAS, protoplast transformation, and microprojectile bombardment. The new knowledge being gained in the field of plant genomics will help breeders make even better use of these techniques to boost the results of agricultural research to the next level. However, to make this all work, the public and private sectors must cooperate with each other and everyone must be cognizant of related biosafety and intellectual property aspects.

References

- 1) Alejar, M. S., Zapata, F. J., Senadhira, D., Khush, G. S. and Datta, S. K. (1995): Utilization of anther culture as a breeding tool in rice improvement. In *Current Issues in Plant Molecular and Cellular Biology*, M. Terzi, R. Cella and A. Falavigna (eds.): Kluwer Academic Pub., Dordrecht, 137-142.
- 2) Alam, M. F., Datta, K., Abrigo, E., Vasquez, A., Senadhira, D. and Datta, S. K. (1998): Production of transgenic deepwater indica rice plants expressing a synthetic *Bacillus thuringiensis cryIA(b)* gene with enhanced resistance to yellow stem borer. *Plant Sci.* 135, 25-30.
- 3) Bennett, J. (2000): Balancing needs for productivity and sustainability: genetic engineering of rice at IRRI. In *Managing Agricultural Biotechnology*, J. I. Cohen (ed.): CABI Publishing, Wallingford, U.K., and the International Service for National Agricultural Research, The Hague, The Netherlands, 184-193.
- 4) Carter, J. (1999): First step toward peace is eradicating hunger. *International Herald Tribune*, 17 June.
- 5) Cohen, M. B., Jackson, M. T., Lu, B. R., Morin, S. R., Mortimer, A. M. and Wade, L. J. (1999): Predicting the environmental impact of transgene outcrossing to wild and weedy rices in Asia. In *Gene Flow and Agriculture: Relevance for Transgenic Crops*, Proceedings of a Symposium: University of Keele, Staffordshire, U. K., 151-156.
- 6) Datta, K., Vasquez, A., Tu, J., Alam, M. F., Oliva, N., Abrigo, E., Khush, G. S. and Datta, S. K. (1998): Constitutive and tissue-specific differential expression of *cryIA(b)* gene in transgenic rice plants conferring enhanced resistance to rice insect pests. *Theor. Appl. Genet.* 97, 20-30.
- 7) Datta, S. K., Peterhans, A., Datta, K. and Potrykus, I. (1990): Genetically engineered fertile indica rice recovered from protoplasts. *Bio/Technology* 8, 736-740.
- 8) Fischer, K. S., Barton, J., Khush, G., Leung, H. and Cantrell, R. (2000): Collaborations in rice. *Science* 290(5490), 279-280.
- 9) Hossain, M., Bennett, J., Datta, S., Leung, H. and Khush, G. (2000): Biotechnology research in rice for Asia:

- priorities, focus, and directions. In *Agricultural Biotechnology in Developing Countries: Towards Optimizing the Benefits for the Poor*, M. Qaim, A. Krattiger and J. von Braun (eds.): Kluwer Academic Publishers (in press).
- 10) IRRI (2000a): *Biotechnology's cutting edge*. In *The Rewards of Rice Research: International Rice Research Institute*, Makati City, Philippines, 20-22.
 - 11) IRRI (2000b): *Molecular breeding en masse*. In *The Rewards of Rice Research: International Rice Research Institute*, Makati City, Philippines: Web version, www.cgiar.org/irri/AR2000/MolecularBreeding.pdf.
 - 12) Jackson, M. (1999): *Managing genetic resources and biotechnology at IRRI's rice genebank*. In *Managing Agricultural Biotechnology*, J. I. Cohen (ed.): CABI Publishing, Wallingford, U.K., and the International Service for National Agricultural Research, The Hague, The Netherlands, 102-109.
 - 13) James, C. (1999): *Global review of commercialized crops: 1999 (Preview)*. ISAAA Briefs No. 12: International Service for the Acquisition of Agri-biotech Applications, Ithaca, New York.
 - 14) Khush, G. S. and Brar, D. S. (1998): *The application of biotechnology to rice*. In *Agricultural Biotechnology in International Development*, C. L. Ives and B. M. Bedford (eds.): CAB International, Wallingford, U.K., 97-121.
 - 15) Khush, G. S. and Leung, H. (2000): *Plant genome research and breeding strategies for sustainable food production in the 21st century*. In *Frontiers of Science and Technology*, L. Esaki (ed.): Universal Academy Press, Tokyo, Japan (in press).
 - 16) Lynne, L. (2000): *Frankenstein Food*. The Straits Times. 29 August.
 - 17) Normile, D. (2000): *Despite the need to feed growing populations in Asia, controversy over GM foods is putting a damper on efforts to develop and test new crops*. Science 289(5483), 1279-1281.
 - 18) Padolina, W. (ed.) (2000): *Plant Variety Protection for Rice in Developing Countries: Impacts on Research and Development*. Limited Proceedings #3: International Rice Research Institute, Los Baños, Philippines. 233 p.
 - 19) Persley, G. J. and Lantin, M. M. (eds.) (2000): *Agricultural Biotechnology and the Poor*. Proceedings of an International Conference, Washington, D.C., 21-22 October 1999: CGIAR and U.S. National Academy of Sciences. 235 p.
 - 20) Pinstrop-Andersen, P. (1999): *The developing world simply can't afford to do without agricultural biotechnology*. International Herald Tribune, 22 October.
 - 21) Polk, P. (2000): *Split Opinions on Gene-Splicing in Foodstuffs*. International Herald Tribune, 25 April.
 - 22) Shimamoto, K., Terada, R., Izawa, T. and Fujimoto, H. (1989): *Fertile transgenic rice plants regenerated from transformed protoplasts*. Nature 337, 274-276.
 - 23) Swaminathan, M. S. (1999): *Perspective: The Challenges Ahead*. New Agriculturist, September.
 - 24) Tanksley, S. D. and McCouch, S. R. (1997): *Seed banks and molecular maps: unlocking genetical potential from the wild*. Science 277, 1063-1066.
 - 25) Tu, J., Datta, K., Khush, G. S., Zhang, Q. and Datta, S. K. (2000a): *Field performance of Xa21 transgenic indica rice (Oryza sativa L.)*. Theor. Appl. Genet. 101, 15-20.
 - 26) Tu, J., Zhang G., Datta, K., Xu, C., He, Y., Zhang, Q., Khush, G. S. and Datta, S. K. (2000b): *Field performance of transgenic elite commercial hybrid rice expressing Bacillus thuringiensis δ -endotoxin*. Nature Biotechnology (in press).
 - 27) Ye, X., Al-Babili, S., Klöti, A., Zhang, J., Lucca, P., Beyer, P. and Potrykus, I. (2000): *Engineering the provitamin A (beta-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm*. Science 287(5451), 303-305.
 - 28) Yoon, C. K. (2000): *A call for biotechnology to be used for the developing world*. The New York Times, 11 July.