



## A recipe for revolution?

Sequencing the DNA of the world's leading food crop was the easy part. Now comes the tricky task of turning our new knowledge of the rice genome into agricultural and economic gains. David Cyranoski reports.

Last year was a good one for rice genomics. Draft genome sequences of the two agriculturally important subspecies of rice, called *indica* and *japonica*, were published in April<sup>1,2</sup>. And in November, the International Rice Genome Sequencing Project (IRGSP) unveiled high-quality sequences of two of *japonica*'s 12 chromosomes<sup>3,4</sup>.

In the wake of these achievements, expectations are high. "Rice DNA finding will transform how the world is fed," is how one British newspaper reported the publication of the two draft sequences. But what do we really know about the rice genome, and its potential agricultural benefits? Not much, admits Takuji Sasaki of the National Institute of Agrobiological Sciences in Tsukuba, Japan, who heads the IRGSP. "We are at the starting line for rice genomics, both basic and applied," he says.

From the standpoint of Sasaki and his IRGSP colleagues, the first stage of this race will involve identifying each rice gene and assigning functions to them. But whereas the sequencing was conducted as an entirely open, team effort, economic considerations may mean that rice functional genomics will become a rather less collaborative venture. Some governments that are investing in the field want to ensure that their own nationals have privileged access to any tools that they develop, and intellectual-property issues complicate the picture still further.

There is no clear finishing line, but the worth of the rice genome will eventually be judged in terms of economic and agronomic gains. This means that different researchers may end up running in different directions. Rich countries such as Japan, for instance, are interested in improving such traits as the taste and texture of the grain. In contrast, plant breeders at the International Rice Research Institute (IRRI) near Manila in the Philippines want to produce higher-yielding or nutritionally superior varieties that will be able to tolerate harsh environmental conditions, in order to improve the lot of impoverished farmers in the developing world.

### Two cultures

What's more, achieving these divergent goals will require genome researchers to begin a meaningful dialogue with breeders in the field. Currently, the different scientific cultures and approaches of the two groups present a formidable obstacle. "These two communities have to get together, but it's like there's a bridge missing," Susan McCouch, who works on molecular approaches to plant breeding at Cornell University in Ithaca, New York, told the International Rice Genome Meeting 2003, held in Tsukuba in February. All in all, it seems that researchers in different camps need to figure out what they want from the rice genome, and how best to get it.

At least the genome researchers are clear on their first move: to work out the functions of all

Rice to the occasion: farmers in poor nations such as Nepal (above) and rich ones like Japan (inset) stand to gain different things from rice genomics.

of the rice genes — a total estimated at around 60,000 by gene-hunting computer programs. About half of these genes have been assigned vague functions on the basis of their sequences — researchers might surmise, for instance, that a gene encodes a member of a particular class of enzymes. But much work remains to be done. "The categories are almost meaningless," says Hirohiko Hirochika of Japan's National Institute of Agrobiological Sciences. So far, only about 100 rice genes have been ascribed a precise, verified function.

The availability of sequence information has already hastened the gene hunt, however. Masahiro Yano, also at the National Institute of Agrobiological Sciences, estimates that the rice genome project trimmed between one and three years off his hunt for a gene that controls flowering time<sup>5</sup>, by providing genetic markers that he could track through breeding experiments to pin down the location of a candidate gene.

Accelerating progress further will depend on new tools, including huge libraries of mutant plants created by randomly inserting tagged bits of DNA into the genome to disrupt their genes. Hirochika has already created 50,000 mutants using this method, whereas Gynheung An at the Pohang University of Science and Technology in South Korea has



made a library of 100,000 plants. In half of An's mutants, the tagged DNA contains a promoter sequence that can boost the activity of nearby genes. An's team has already created plants in which growth is stunted or flowers bloom late, as well as mutants with increased sensitivity to heavy metals or salts<sup>6</sup>.

In addition to randomly created mutants, rice geneticists would like to develop the ability to knock out specific genes at will. A new tool developed by Shigeru Iida and his colleagues at the National Institute for Basic Biology in Okayama, Japan, could provide the answer<sup>7</sup>. It exploits the occasional tendency of DNA to insert itself into the genome at points where the sequence matches its own — a phenomenon known as 'homologous recombination' — to disrupt the function of particular target genes.

DNA microarrays will also speed the analysis of gene function. Microarrays can carry tens of thousands of genes or predicted gene sequences, allowing quick screens of a plant's genetic make-up, or the activity of its genes, to be carried out. A chip made by the multinational agribiotech firm Syngenta has already been used to identify 269 genes that are expressed when a rice grain is being filled up with its store of carbohydrates, proteins and fatty acids<sup>8</sup>. Microarrays are especially useful because they can reveal the activity of networks of genes, which are extremely difficult to study by random mutation or targeted gene knockouts.

But access to such tools is not completely open. Syngenta's microarray is a proprietary technology, and Iida's gene-targeting technique is similarly being patented. Newly discovered genes may also be patented as they are discovered. "Intellectual property came into rice research with genomics," observes IRRI director general Ron Cantrell.

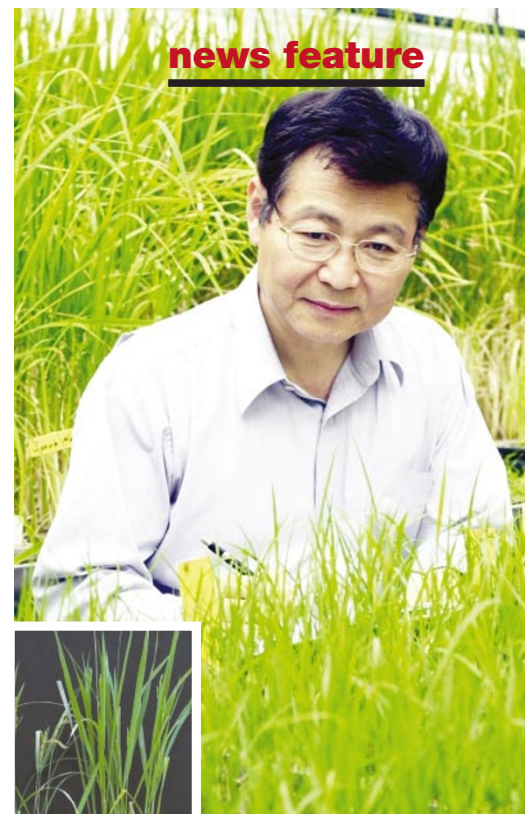
Even if patents do not restrict access to important functional-genomic tools, such resources may not be immediately available

to all researchers. Japan this month launched a programme to assemble a rice genome resource centre at the National Institute of Agrobiological Sciences, but most of its mutants, microarrays and other tools will not be released for a couple of years — and even then, Japanese researchers will get their hands on them first, giving them a head start on work that may prove to be economically valuable. Indeed, most nations with an interest in rice research, including China, are now assembling their own functional-genomics resources to ensure that they are not left at a disadvantage.

### Genome duplication

This competition and parallelism of effort is making some experts uneasy. "Not everyone needs a microarray or knockout programme," argues Hei Leung, a plant pathologist at IRRI who is trying to convince key players to combine their resources for the greater good of both research and agriculture. Leung has sown the seeds of an international functional-genomics consortium involving 20 members from 17 institutions around the world, and he aims to form a resource centre that by 2010 will hold mutants for 90% of all rice genes, of which 60% will have been functionally analysed.

Many plant breeders remain sceptical about the value of genomic information, however. The most important contribution so far has been the provision of genetic markers, DNA sequences located near genes that can be tracked to breed for traits that are difficult to observe — a process called 'marker-assisted selection'<sup>9</sup>. There are markers, for example, for more than 20 rice genes that confer resistance to a disease known as bacterial leaf blight. Breeders would like to combine many of these genes for maximum effect, but the effect of a certain gene might be slight in the particular testing environment. The presence of the markers, which



Among the mutants created by Gynheung An (above) is a rice plant (left) that flowers later in the growing season.

can be easily verified, can tell a breeder that a trait gene is present.

But markers can frustrate breeders by becoming detached from the gene when chromosomes recombine during breeding crosses. This is especially true for complex traits, such as drought resistance, in which many genes may be involved. "Markers can be hard to use with confidence," says David Mackill, head of plant breeding at IRRI.

Having more markers would increase the chance of finding one that is tightly linked to the gene for a particular desirable trait. With this in mind, a consortium led by McCouch and researchers at IRRI is aiming to increase the number of markers available.

What about the provision of new information on the function of rice genes? Such knowledge will be welcome, but simply knowing the location of a given gene and the effect of its disruption in a mutant plant is of limited value to plant breeders. They are more interested in knowing what natural variants, or alleles, the gene has — and how to recognize these alleles in crosses. "Too often, gene-discovery studies do not take the work to the resolution that is really needed for applications in breeding," says McCouch.

She is trying to go the extra mile to make genomic information more useful to breeders. At the February meeting in Tsukuba, McCouch described her analysis of the gene that underpinned the 'green revolution' of the 1960s by producing dwarf rice plants that



Full steam ahead: will rice breeders use genomic knowledge to generate new varieties for field tests?





Mister rice guy: genomics could help to ensure that rice distributors' varieties are the genuine article.

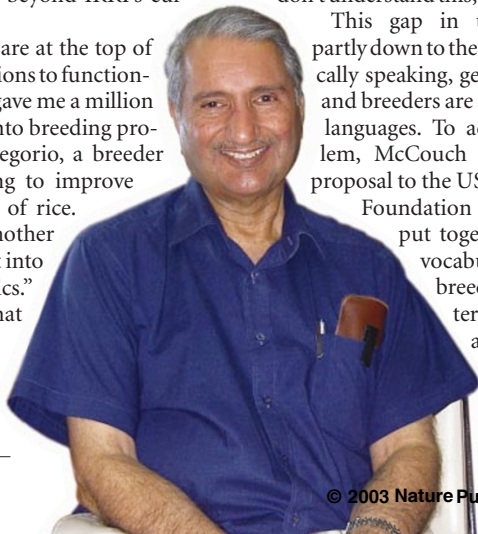
put more of their energy into making grain, rather than stems. The precise identity of the gene was finally revealed last year<sup>10</sup>, and McCouch has now developed a method that can differentiate between its individual alleles. After listening to McCouch's talk, one excited breeder in the audience said: "That's exactly what we need from the genome."

### Tracking traits

Microarrays might also be used to screen for useful new alleles. IRRI alone has collected 100,000 varieties of rice from around the world — most of these are not good crop plants, but they are thought to hold many unknown alleles that might be bred into existing varieties to useful effect. Microarrays spotted with genes that are known to be of agronomic significance could take much of the work — not to mention guesswork — out of identifying potentially valuable alleles. But it would not be cheap — with microarrays costing around US\$400 apiece, a comprehensive screen would be way beyond IRRI's current resources.

In fact, high costs are at the top of breeders' list of objections to functional genomics. "If they gave me a million dollars, I'd put it all into breeding projects," says Glenn Gregorio, a breeder at IRRI who is trying to improve the nutritional value of rice. "Maybe if I had another million, I would put it into functional genomics." But the reality is that

**Gurdev Khush: rice breeders still have to find the right gene combinations.**



funding for rice research at IRRI and elsewhere is extremely tight.

Sometimes, even knowing an allele and its markers may not be enough. Yano's flowering-time gene, a natural variant, offered breeders an allele with which to control the timing of harvest. But attempts to breed the allele into commercial varieties of rice have proved a big disappointment. "They taste bad," says one breeder at a regional Japanese agricultural station. "It hasn't really been any use."

This example neatly illustrates the difficulty of turning genomic information into agronomic advances. "Gene identification is one thing, but combining the genes as required is another," says Gurdev Khush, now at the University of California, Davis, and formerly of IRRI, where he developed 308 varieties of rice for release during his career. No matter how closely a gene is studied in the lab, it is down to the breeder to discover how it will function in different varieties, or when faced with environmental stresses such as drought and high salinity. "Most molecular biologists don't understand this," says Cantrell.

This gap in understanding is partly down to the fact that, scientifically speaking, genome researchers and breeders are speaking different languages. To address this problem, McCouch has submitted a proposal to the US National Science

Foundation for funding to put together a 'controlled vocabulary' to help breeders, who talk in terms of traits and alleles, communicate more effectively with the geneticists, who focus on genes

and pathways. At present, the two groups even use different yardsticks to measure distances along chromosomes.

IRRI, which in recent years has seen its efforts overshadowed by the publicity generated by rice genomics, hopes to play a key role in bridging the gap between genome researchers and plant breeders. This will be difficult, however, given the diverse demands placed on breeders for varieties of rice that are suited to specific environments — highly productive irrigated areas, lowland areas that receive some rain, flood-prone regions, upland areas, and so on. Depending on the location, breeders might need to incorporate into native varieties traits such as drought resistance, submergence resistance, disease resistance and salinity tolerance.

### Mass catering

To meet these diverse needs, IRRI may have to take a step back from trying to deliver finished products, and instead serve up functional-genomics tools, alleles and markers that can be used by local breeders to address their various needs. "We have to supply information," says Cantrell.

Another problem is that, with investment in rice genomics being dominated by Japan and other developed countries, many of the functional-genomic tools now being developed are optimized for their favoured *japonica* subspecies. Inevitably, this will restrict progress in analysing the genome of *indica*, the staple that feeds most of the rice-eating world.

In affluent Japan, the basic agronomic traits that are of primary concern to breeders in developing countries are often of secondary importance to the question of eating quality. Recently, for example, breeders in the Fukui region came up with a set of markers for the gene that gives the desired 'stickiness' to the popular *koshihikari* variety of *japonica*, in the hope that these will help in breeding the trait into other varieties. In a separate endeavour, a company called Plant Genome Center in Tsukuba has developed a set of markers to help to unmask producers who are attempting to pass inferior varieties off as *koshihikari*.

Despite the complexities involved, enthusiasts for rice genomics remain confident that the investment in the rice genome will eventually yield a valuable harvest. But those who were led by some of last year's headlines to expect an imminent agricultural revolution will have to learn to be patient. ■

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