GENE HETTEL (2)

Even after 17 days of submergence in IRRI research plots, Sub1 rice lines show their "waterproof" trait as they are still standing to the left, right, and further behind IRRI plant physiologist Abdel Ismail.

Scuba rice

Stemming the tide in flood-prone South Asia

by **Adam Barclay**

New versions of popular varieties of rice, which can withstand 2 weeks of complete submergence, are set to make a big impact in South Asia

Scientists had long known of an Indian rice variety, unromantically dubbed FR13A, that could handle a week or more of complete submergence and recover sufficiently to offer a reasonable harvest. Rice, although often grown in standing water, will drown like any other plant if hit with severe flooding.

Despite its remarkable properties, FR13A (FR stands for "flood resistant"), as a low-yielding traditional variety grown across limited areas in the Indian state of Orissa, was never expected to make a big impact on a wide scale. Nevertheless, rice breeders—including David Mackill, a young Californian plant breeder working at the International Rice Research Institute (IRRI) in the 1980s—saw the potential to breed FR13A's sought-after trait into some of the modern high-yielding rice varieties planted over vast flood-prone areas across Asia.

His reasoning, which emerged from discussions with IRRI deepwater rice breeder Derk HilleRisLambers, was that a flood-tolerant version of a popular modern variety could have an enormous impact. In Bangladesh and India, for example, farmers suffer annual crop losses because of flooding of up to 4 million tons of rice—enough to feed 30 million people. To the farm families and workers, and to the poor consumers who rely on rice for the bulk of their food, flooding can be truly disastrous.

So, the IRRI breeders—people who spend their careers mixing the genes of plants to develop new varieties that can handle harsh climates, or resist diseases and pests,

IRRI PLANT breeder Dave Mackill (*right*) swaps notes at BRRI's Rangpur station with UC Davis Professor Pam Ronald.

or cope with problem soils—tried. And they succeeded. Sort of. They created higher-yielding rice plants that could handle major floods, but they never even got close to releasing them to farmers. During the breeding process, which transferred to the modern varieties whichever genes were giving FR13A its flood tolerance, too many unwanted genes moved across as well. The result was poor-tasting, flood-tolerant rice that yielded no more than existing varieties. And so the idea moved to the back burner.

In 1991, Dr. Mackill left IRRI for the University of California (UC) at Davis. With FR13A still on his mind, he and his graduate student Kenong Xu took up the challenge of identifying the genes responsible for FR13A's scuba abilities. They eventually pinpointed the precise stretch of DNA that made the variety so interesting, and named the assumed gene *SUB1*.

The group subsequently teamed up with another UC Davis researcher, Pamela Ronald, an expert in isolating genes that give plants particular traits. Working in Dr. Ronald's lab, Dr. Xu and his wife, Xia, discovered a single gene, which they named *SUB1A*, and demonstrated that this alone was responsible for most of the flood tolerance.

Dr. Mackill, who by now had returned to IRRI, realized that the FR13A game was back on. By that time, 25 years after the first breeding attempts, agricultural science had come a long way. A new "precision-breeding" method, known as marker-assisted selection (MAS; see *On your mark, get set, select* on pages 28-29 of *Rice Today* Vol. 3, No. 3; also see *From genes to farmers' fields* on pages 28-31 of *Rice Today* Vol. 5, No. 4), allowed breeders to do much of their work in the lab. The new method shortened the breeding process and vastly improved the precision with which specific traits could be moved from one variety into another. He and his team were able to transfer

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DR. ISMAIL and UC Riverside scientist Julia Bailey-Serres share a laugh at BRRI's Rangpur station.

SUB1A into widely grown modern rice varieties without affecting other characteristics—such as high yield, good grain quality, and pest and disease resistance—that made the varieties popular in the first place.

By 2006, the first Sub1 varieties were ready for testing at IRRI. The researchers set up plots of what they hoped would be flood-tolerant versions of several varieties—IR64, Swarna, and Samba Mahsuri—next to plots of their non-Sub1 counterparts. Once the plants had established themselves, the plots were flooded, completely submerging the rice for 15 days. Next, the water was drained to reveal muddy plots of limp, flattened, deathly looking plants.

Then, a remarkable thing happened. Within 2 weeks of the flood, almost all of the Sub1 plants recovered. They came back to life as if coached by Lazarus[1] himself. A few scattered clumps of the original versions made a comeback, but there was no comparison. At harvest, the Sub1 rice yielded more than twice as much as its neighbor (to view a dramatic time-lapse video of the experiment, visit http://snipurl.com/ebql8).

Around the same time, following Dr. Ronald's group's success in proving that *SUB1A* was indeed the right gene, Julia Bailey-Serres, a geneticist from UC Riverside who also worked on the gene's identification, began investigating exactly *how* *SUB1A* confers flood tolerance. It turns out that the secret is all about saving energy.

With colleague Takeshi Fukao, Dr. Bailey-Serres has determined that, when submerged, rice without *SUB1A* responds by increasing the pace of its elongation in an attempt to escape the submergence. Deepwater rice varieties are able to do this rapidly enough to succeed. In modern high-yielding varieties, however, the elongation is insufficient. If the flood lasts for more than a few days, the normal varieties expend so much energy trying—unsuccessfully—to escape that they're unable to recover.

Submergence of FR13A or any of the new Sub1 varieties, on the other hand, activates the *SUB1A* gene, which suppresses this elongation strategy, effectively shunting the rice plant into a dormant state until the floodwaters recede. Thus, the plants conserve their energy for a postflood recovery.

"Understanding things from this very basic perspective should allow us to achieve an even better plant more rapidly," says Dr. Bailey-Serres.

According to Dr. Mackill, the Sub1 project has shown the advantage of combining practical, applied work such as breeding and upstream, fundamental research.

""Knowing the exact gene responsible for a trait is not absolutely necessary for the MAS breeding approach, because a larger piece of the chromosome is transferred, normally containing many genes," he says. "However, by understanding the processes triggered by *SUB1A* in detail, "we hope to improve on the existing Sub1 varieties by identifying novel flood-tolerance genes that allow us to develop hardier plants that survive even longer periods of

[1]A Biblical name used to connote apparent restoration to life.

flooding, yet retain the characteristics that farmers want."

With the Sub1 concept well and truly proved, seeds were sent for testing and refinement to national organizations in South Asia, including the Bangladesh Rice Research Institute (BRRI) and, in India, the Central Rice Research Institute (CRRI) in Orissa and Narendra Dev University of Agriculture and Technology in Faizabad, Uttar Pradesh. The trial results there were also extremely promising.

In short, scientists had developed rice that could handle more than a week's flooding with almost no loss of yield (1 week is enough to severely dent the harvest of the nontolerant versions) and would recover to produce a reasonable yield after even 2 weeks' submergence (enough to almost wipe out nontolerant versions). Aside from the flood tolerance, the new varieties were virtually identical to their counterparts: farmers would be able to manage them in exactly the same way and, in the absence of flooding, achieve the same yield.

But, as any agricultural scientist will tell you, there is a vast gulf between the tightly controlled environment of the experiment station and the more capricious nature of a real farm. By 2007, the time had come to test the Sub1

BRRI DIRECTOR General Mohammad Firoze Shah Shikder (*left*) and BRRI scientist M.A. Mazid explain the flood-tolerant rice trials carried out at BRRI's Rangpur station.

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BRRI SCIENTIST M.A. Mazid (*second from right*) speaks to onlookers about the success of farmer Mostafa Kamal's (*right*) flood-tolerant rice trials. Mr. Kamal's neighbor, Mohammad Shahidul Islam (*left*), is keen to grow the new varieties himself.

varieties in farmers' fields. In this setting, there was no way of controlling when flooding would occur, how long it would last, or whether it would even happen at all.

Moving forward to November 2008, to a small farm in Rangpur District in northwestern Bangladesh, researchers from IRRI, UC, and national institutes in India and Bangladesh commenced a South Asian tour to mark the completion of the project *From genes to farmers' fields: enhancing and stabilizing productivity of rice in submergence-prone environments*, funded from 2004 to 2008 by Germany's Federal Ministry for Economic Cooperation and Development (BMZ).

If ever there was a country with flooding problems, Bangladesh is it. More than 1 million hectares—20%—of the country's rice lands are flood prone.

"In those areas where flooding occurs once or twice and recedes within 12–14 days," says BRRI Principal Scientific Officer M.A. Mazid, who has overseen the Sub1 trials at BRRI's Rangpur station, "the Sub1 varieties could survive and improve yields by up to 3 tons per hectare."

Given that Bangladesh is forced to import around 2 million tons of rice each year, BRRI Director General Mohammad Firoze Shah Shikder says that successful flood-tolerant rice could substantially reduce, if not eliminate, the country's imports.

"Sub1 varieties will add to the total production of the country," he says. "They will save a lot of money that would otherwise be used for importing rice."

Moreover, within that single, large-scale outcome, there would be thousands and thousands of equally positive, smaller-scale achievements. Many farm families, eking out a living on less than a hectare, could ensure that they had enough rice to eat year-round. Others would harvest enough to sell their surplus on the market and increase their income.

Mostafa Kamal is one of the farmers BRRI recruited to test the Sub1 varieties in his field. He and his brothers have a 6-hectare farm—large by Bangladeshi standards—that needs to produce enough rice each year to feed 22 members of the Kamal family. The farm suffers heavy losses because of flooding every 4 out of 5 years.

"In the past, many of my plots became fallow because they were flooded too often," says Mr. Kamal, referring to the lowest-lying 2 hectares of the farm. "If we can cultivate on these plots, it will help us produce rice to sell on the market.

Two extra hectares is a big jump."

So, how did the flood-tolerant varieties fare? Twenty-three days after the 8 July transplanting of the 2008 wet-season crop, the farm was hit by a 15-day flood. When the waters receded, Mr. Kamal witnessed a wonderful thing. In his Sub1 plots, 95–98% of the plants recovered. In the non-Sub1 plots, the figure was 10–12%. Many of his neighboring farmers, who were not involved in the trial, lost their entire crops. So encouraged was Mr. Kamal, he planned to give away—not sell—a kilogram of flood-tolerant seeds to each of his neighbors.

"When I saw Mostafa's field flooded, and then saw it recover, I was surprised—it was like magic," recalls Mr. Kamal's neighbor, Mohammad Shahidul Islam. The annual flash floods mean that Mr. Islam grows rice on only the upper half of his 1.6-hectare farm in the wet season. Each year, he needs to buy 1 to 2 months' worth of rice to cover his family's shortfall. He believes that flood-tolerant varieties will allow him to plant on his low-lying 0.8 hectare and cover that shortage. "These varieties,"

FOLLOWING A 10-DAY flood, Orissa farmer Bidhu Bhusan Raut saw his Swarna-Sub1 recover well while his nontolerant Gayatri perished. "Better yielding is better living," he says.

he says, "will mean more food, higher income, and a better livelihood."

Observing the success of the flood-tolerant varieties in Bangladesh was a watershed moment for Sigrid Heuer, an IRRI molecular biologist who contributed to the analysis of *SUB1A*.

"I knew all along *SUB1A* was working in any type of rice we put it in," she says. "I've seen it many times at IRRI and I've seen the data from the field experiments in India. But I'd never seen it in farmers' fields with my own eyes. Here, I've seen it after natural flooding for 15 days—the maximum time we think *SUB1A* should be able to withstand—and it's working. It's really fantastic."

A short flight away in eastern India, it is the same story. The states of West Bengal and Orissa, along with Uttar Pradesh in the northeast, have all seen equally promising trial results and plan to completely replace Swarna with Swarna-Sub1 as soon as it is officially released by state seed certification agencies. In West Bengal, Swarna dominates, with 80% of the rice area already planted to the variety. A move to Swarna-Sub1 would therefore be relatively easy and stands to have enormous impact.

"Forget Swarna! Go for Swarna-Sub1!" is the advice from Basant Kumar Rao, a rice farmer from Nuagaon Village near Cuttack in Orissa. "I trust Swarna-Sub1. I'll keep growing it. I got good money for it in 2007," he says.

That year, his farm was hit by two floods, one of 11 days and one of 7 days. The flood-tolerant rice recovered after both floods and, although he was able to salvage a little of his regular Swarna, it yielded nowhere near as well.

"Better yielding is better living," according to another Orissa farmer, Bidhu Bhusan Raut. In the 2008 wet season, Mr. Raut grew Gayatri, a popular Indian variety, and Swarna-Sub1 on his entire 1-hectare farm.

"FORGET SWARNA! Go for Swarna-Sub1!" says Basant Kumar Rao, a rice farmer from Nuagaon Village near Cuttack in Orissa. Here, he stands in his crop of Swarna-Sub1, which recovered well after two floods hit his farm in the 2007 wet season.

THE DEVELOPMENT and testing of flood-tolerant rice varieties—on show here at BRRI's Rangpur station—have attracted keen interest from plant scientists across the world.

After a 10-day flood, the Sub1 plants recovered well, while the Gayatri plants perished.

According to CRRI Director T.K. Adhya, the release of flood-tolerant rice has become more and more important as India has grown economically.

"People used to grow rice in more favorable areas, where you had an assured source of water and good soil quality," he explains. "Now, those interior areas are being taken over by human habitation and industry, so farmers are forced onto marginal lands in the coastal areas where flooding, salinity, and many other problems occur. In the past, farmers simply had to face flooding and blame their luck if they didn't get a harvest."

IRRI plant physiologist Abdel Ismail, who is studying the mechanism of *SUB1A*'s action, says there is a strong case for rapid release of the new varieties.

"When you develop varieties using marker-assisted selection," he says, "you do not change the variety much. Because the *SUB1A* gene is very specific in its expression and action during submergence, the Sub1 varieties should not have any other problems—such as susceptibility to diseases or insects—that their nontolerant counterparts wouldn't have also. In the future, we expect many new varieties to come out as products of MAS. If you have a submergence-tolerant or salt-tolerant variety, for example, you want it to go to the field as quickly as possible, where it can make a big difference."

N. Shobha Rani, principal scientist at India's Directorate of Rice Research, says that traditionally bred rice must undergo testing for 3 years in all-India trials, but this has been reduced to 2 years for MAS-derived varieties.

"The second year of testing is 2009," says Dr. Rani, "so, April 2010 is the earliest time the Sub1 varieties could be recommended by the Central Variety Release Committee for national release." She notes, however, that release could occur on a state basis before then.

In fact, on 27 February 2009, only a few months after Dr. Rani talked to *Rice Today*, the Uttar Pradesh State Varietal Release Committee officially released Swarna-Sub1. Being nearly identical—apart from its flood tolerance—to Swarna, this inaugural release of a Sub1 mega-variety occurred very quickly: only 6 years after the first cross was made at IRRI.

A quick release is also possible because plants developed through MAS are not transgenic (that is, genes of interest are transferred to the target species or variety using particular biotechnological tools rather than conventional breeding). Therefore, the new Sub1 varieties are

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EXAMINING TRIALS at BRRI headquarters in Gazipur, K.M. Iftekharuddaula (*right*) has bred flood tolerance into popular Bangladeshi rice variety BR11, which accounts for more than one-third of the country's wet-season plantings.

A PATCH of the popular rice variety Swarna lies flattened and dying after several days of flooding. In contrast, the flood-tolerant version, Swarna-Sub1, rebounds to good health.

not subject to the regulatory testing that can delay release of transgenic products for several years.

The Sub1 trait also came along with an additional bonus, a gene linked to *SUB1A* that turns the normally golden color of the hull of Swarna into a straw color. Although the hull color is not considered an important varietal requirement, this allows the seeds of Swarna-Sub1 to be easily distinguished from those of Swarna. This will be useful to maintain seed purity as seed producers start ramping up the production of foundation seed for distribution to farmers.

Another success to emerge from the Sub1 work has been the strengthening of national organizations such as BRRI and CRRI.

"In India now, MAS has a lot of support from the government," says Dr. Ismail. "In Bangladesh, BRRI has its own lab for MAS, and not just for *SUB1*. In the national agricultural research and extension systems, the project has boosted capacity through resources and expertise, and also through government support."

BRRI researcher K.M. Iftekharuddaula is a good example. He carried out his Ph.D. research under Dr. Mackill's supervision at IRRI headquarters in the Philippines, developing a flood-tolerant version of popular Bangladeshi variety BR11, which accounts for more than one-third of the country's wet-season plantings. After completing his thesis research, he returned to Bangladesh, where he is now the BRRI breeder responsible for refining BR11-Sub1 varieties for official release.

"We are very much hopeful that we'll be able to release at least two varieties from our efforts," says Mr. Iftekharuddaula, who is also working with IRRI to incorporate disease resistance and salinity tolerance into BR11-Sub1.

As Sub1 varieties are officially released over the next 2 years, the key will be dissemination to smallholder farmers in flood-prone areas. IRRI is leading this initiative through the project *Stress-Tolerant Rice for Poor Farmers in Africa and South Asia*, funded by the Bill & Melinda Gates Foundation. IRRI is also collaborating with national organizations to test Sub1 varieties in Southeast Asian countries, including Laos, Thailand, Cambodia, Indonesia, Vietnam, and the Philippines, through a project funded by Japan's Ministry of Foreign Affairs.

Dr. Ismail adds that *SUB1A*'s effectiveness offers hope for research into tolerance of other so-called abiotic stresses, such as drought and salinity.

"The general notion with abiotic stresses used to be that it would be very difficult to find a single gene that can make much difference," he says. "This work has shown that you can get a single gene of great agronomic value. I think this has set the tone for solving other major difficulties in the field, such as problem soils."

The story of the *SUB1* research underscores the capacity of science to improve people's lives, as well as the power inherent in a gene. It seems a long and unlikely journey from experimental plots in the Philippines and the laboratory benches in California to a small farm in Bangladesh.

For Drs. Ronald and Bailey-Serres, the chance to get out of the lab and see the Sub1 varieties in farmers' fields has been a profound experience.

"It was amazing to see that this detailed genetic and physiological analysis ultimately has potential for a grand impact on people who are often living in pretty desperate situations," Dr. Bailey-Serres says.

Even Dr. Heuer, who, through her work at IRRI, is no stranger to Asia's rice fields, has been moved. "I had no idea about the impact we can have before seeing it with my own eyes," she adds. "I've learned about the power of agricultural research here. I think it will have a huge impact."

Mr. Barclay is a freelance writer based in Australia. See www.irri.org/flood-proof-rice.

IRRI MOLECULAR biologist Sigrid Heuer (*center*) with her Ph.D. student Namrata Singh (*left*) and IRRI assistant scientist Darlene Sanchez at the Chinsurah Rice Research Station, in West Bengal, India.