



# From genes to farmers' fields

by David Mackill

*The practical application of gene discovery to develop submergence-tolerant rice will help farmers avoid the ravages of severe flooding*

Rice is considered a semiaquatic plant, and it thrives in the wettest agricultural environments. However, most rice varieties will be heavily damaged and die if they remain underwater for more than 4 days. A few varieties—such as the traditional Indian variety FR13A—can tolerate 2 to 3 weeks of submergence and rapidly recover when the water subsides. This is important for the vast rainfed lowland areas of Asia where intermittent flooding causes frequent submergence. Estimated crop losses are around a billion dollars annually. Compounding the problem, submergence stress tends to be more common in areas where poverty is

high. Rice production in these areas is highly variable due to flooding. Relief may be at hand, though. Researchers at the International Rice Research Institute (IRRI) and the University of California (UC Riverside and Davis) have recently discovered a gene from FR13A that is responsible for that variety's strong tolerance of submergence. This gene belongs to a class of genes known as "ethylene response factors" or ERFs. The significance of the research—recently reported in the scientific journal *Nature*<sup>1</sup>—is that it provides new tools to develop rice varieties that combine high yields and

tolerance of temporary submergence. Submergence-tolerant varieties have been known for a long time, and submergence tolerance has been a breeding focus at IRRI since the early 1970s. In India, varieties like FR13A (FR stands for flood resistant) were selected from farmers' varieties as early as the 1940s and showed a high level of submergence tolerance compared with other varieties. FR13A and other tolerant varieties were crossed with high-yielding semidwarf varieties (which are shorter than most traditional varieties and thus resistant to damage from rain and wind), producing short varieties with

IRRI RESEARCHERS Abdelbagi Ismail (left), Sigrid Heuer (second from right), and Dave Mackill (right) examine a submergence-tolerance screening experiment in an IRRI greenhouse while associate scientist Alvaro Pamplona (center) and Gina Vergara look on. Top left, assistant scientists Jessica Rey (left) and Darlene Sanchez prepare DNA samples for analysis in the lab.

tolerance of submergence. Despite continuing efforts by rice breeders, these varieties were not adopted by farmers because of unacceptable traits such as poor taste or inadequate adaptation to the location.

Work on the genetics of submergence tolerance began in the early 1990s. Graduate student Kenong Xu and I—then working for the Agricultural Research Service of the United States Department of Agriculture and UC Davis—used molecular markers (a molecular marker is a segment of DNA that is linked to a gene that controls an important trait and can easily be detected in the lab) to map a region of DNA on rice chromosome 9 that was responsible for most of the tolerance. We named the gene (or genes—at the time, we didn't know whether it was a single gene or several linked genes) *Sub1* (*Submergence1*). This began the search to pinpoint the genes responsible for submergence tolerance.

We were then joined by Pamela Ronald, an associate professor at UC Davis. Ten years of hard work by Dr. Xu plus a strong contribution from his wife, Xia Xu, would ultimately unravel the DNA sequence of the *Sub1* region. The group was joined by molecular biologists Julia Bailey-Serres and Takeshi Fukao at UC Riverside and Sigrid Heuer at IRRI, who helped analyze the genes in the *Sub1* region (see *Identifying the submergence-tolerance gene*, page 30). IRRI plant physiologist Abdelbagi Ismail and his team have been studying the mechanism of action of *Sub1* (see *The mechanics of submergence tolerance*, page 31) as well as management options for the new submergence-tolerant varieties.

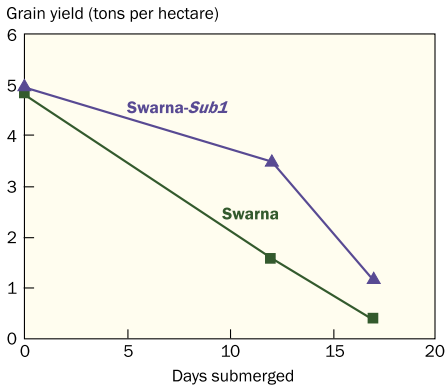
Parallel to the gene-hunting work is research using marker-assisted selection (see *On your mark, get set, select!* on pages 28-29 of *Rice Today* Vol. 3, No. 3) to develop new submergence-tolerant varieties by introducing *Sub1* into widely grown varieties. Using marker-assisted selection, breeders can selectively transfer a small chromosome fragment containing a beneficial gene or genes, while leaving the rest of the genes untouched. For example, the IRRI team has developed a version of the widely

grown variety Swarna—grown on more than 5 million hectares in India and Bangladesh—that contains the chromosome segment containing the *Sub1* gene, while the genes in all other chromosomal regions are those of Swarna. This ensures that the submergence-tolerance trait is added without changing other desirable properties—such as high yield, acceptable taste, and good regional adaptation—of the recipient variety.

The IRRI group has produced submergence-tolerant versions of three widely grown rice varieties: Swarna and Samba Mahsuri from India, and IR64 from IRRI. Three more are near completion: Thadokkham 1 from Laos, CR1009 from India, and BR11 from Bangladesh—each currently grown widely in their respective countries. These new varieties do not show any differences from the originals except for the submergence tolerance. Experiments at IRRI showed that the *Sub1*-enhanced version of Swarna achieves the same yields as "regular" Swarna under normal shallow-water conditions (about 5 tons per hectare), but, when subjected to 12 days of submergence about 4 weeks after planting, followed by a return to shallow conditions, Swarna-*Sub1* has more than double the yield of Swarna



RICE FARMERS from Kaudikol village in the Indian state of Orissa wait for floodwaters to recede. This area experiences flooding as deep as 2.5 meters for up to 3 weeks each year.



**YIELD VERSUS TIME** submerged for "regular" Swarna and Swarna-Sub1. Plants were completely submerged 14 days after transplanting of 14-day-old seedlings in field plots at IRRI.

(3.5 tons per hectare versus 1.6 tons per hectare—see figure above). Swarna-Sub1 was grown at research stations in India and Bangladesh in 2005 and is being tested in farmers' field experiments in 2006.

Dr. Mackill is the head of IRRI's Plant Breeding, Genetics, and Biotechnology Division

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## Identifying the submergence-tolerance gene

by Sigrid Heuer

**M**arker-assisted selection can be implemented at different levels, like a map can be drawn at different scales or resolutions. That means, in molecular terms, that the higher the resolution of the genetic map the closer we are to the actual gene responsible for a particular trait—submergence tolerance in this case. Being very close to the responsible gene is important for our breeding approach. Because we are transferring tolerance

to widely grown and well-adapted varieties, we don't want to change them in any way other than to make them submergence-tolerant. Close markers allow us to develop plants that carry only the tolerance gene, and that remain otherwise identical to the variety that farmers know and like. In the case of *Sub1*, we went down to the highest resolution possible by sequencing the DNA in the chromosomal region where we expected the submergence-tolerance gene to be located.

Within the *Sub1* region, we found several genes and needed to analyze all of them to determine the actual submergence-tolerance gene. By testing the expression of these genes and their response to submergence stress, we narrowed down the number of candidate genes to three. These three genes—*Sub1A*, *Sub1B*, and *Sub1C*—all belong to the same type of regulatory genes, known as ethylene response transcription factors (ERFs). Transcription factors can switch the expression of other genes on or off and therefore often have important regulatory functions.

We then discovered that some intolerant varieties have only two copies of these ERF genes (*Sub1A* is absent) and that tolerant rice varieties have *Sub1A* and *Sub1C* genes that have slightly different sequences than the same genes in intolerant varieties. This pointed to *Sub1A* as the major determinant of submergence



**I WILL SURVIVE:** the one remaining live plant comes from a line derived from Swarna-Sub1's submergence-tolerant parent variety, FR13A. This is part of a new experiment to identify novel submergence-tolerance genes.

SIGRID HEUER

tolerance. However, we needed more evidence. We therefore tested whether or not *Sub1A* could confer tolerance of submergence to previously intolerant plants. To do this, our partners at the University of California, Davis, introduced the tolerant form of the *Sub1A* gene into the intolerant rice variety Liaogeng and, indeed, the plants produced were submergence-tolerant. These plants now provide a very useful tool to study the function of the *Sub1A* gene in detail.

Our partners in UC Riverside have compared the expression of genes in rice plants with and without the *Sub1* locus and we have learned a lot about the genes that are regulated by *Sub1*. In the future, we also need

DAVE MACKILL



**POPULAR RICE VARIETIES** containing the submergence-tolerance gene will be much more likely to survive floods like this one in Cambodia.

to study the genes that regulate *Sub1*.

We already know that when tolerant varieties are submerged, *Sub1A* is induced and *Sub1C* is suppressed. Interestingly, when intolerant varieties are submerged, we see the opposite: *Sub1A* is highly detectable and *Sub1C* expression is high. It therefore seems that a balance of *Sub1A* and *Sub1C* expression is important for tolerance (in addition to differences in the sequence). We now want to identify the genes that determine when and to what extent *Sub1A* and *Sub1C* are expressed.

The identification of tolerance genes and their interaction with other genes is important because it helps us to better understand the underlying mechanisms and the regulation of stress tolerance. Certain genes probably have important functions in other stresses as well, such as drought or salinity. Intimate knowledge of the genetics behind tolerance of, or susceptibility to, these stresses might one day allow breeders to develop multi-stress-resistant rice varieties by adding to established varieties a few key regulatory genes. *Sub1A* will surely be one of these.

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*Dr. Heuer is a molecular biologist in IRRI's Plant Breeding, Genetics, and Biotechnology Division.*

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## The mechanics of submergence tolerance

by Gina Vergara and Abdelbagi Ismail

**A** submerged rice plant faces several problems ranging from inadequate growth to damage and death. For example, low light levels inhibit photosynthesis, slower gas exchange results in lower carbon dioxide intake for photosynthesis and lower oxygen intake for respiration, and gases trapped in the water, such as ethylene, promote plant elongation and chlorosis (yellowing or discoloration of the normally green parts of the plant), with a



AN IRRI FIELD experiment to evaluate Swarna (left) and Swarna-Sub1 (right), which carries the submergence-tolerance gene. Fourteen-day-old seedlings were transplanted in the field and, 14 days later, submerged for 12 days. Shown here 60 days after transplanting, Swarna-Sub1 is doing well while few Swarna plants have survived.

SUDHANSHU SINGH

consequent loss of photosynthetic area. Therefore, our research team examined the mechanics that allow rice plants with the *Sub1* genes to tolerate submergence.

The Indian variety FR13A can survive submergence because of some key physiological traits that emerge when the plant is underwater. FR13A maintains a higher carbohydrate supply in its shoots before submergence and does not greatly elongate, meaning it conserves energy that can then be used for survival. It also experiences slower carbohydrate depletion and higher rates of alcoholic fermentation as a way of providing energy for maintenance processes in the absence of oxygen underwater. And, when submerged, FR13A maintains higher chlorophyll levels—allowing relatively better photosynthesis during submergence and also after the water recedes to resume its growth and recovery—than intolerant varieties.

As survival strategies, maintenance of higher carbohydrate levels before and during submergence and minimization of both elongation growth and chlorosis had the most striking effects. To demonstrate this, our team inhibited the synthesis of ethylene—which reduces its chance of affecting a submerged plant's survival—in submerged conditions. This resulted in an improved survival rate for submergence-intolerant varieties but not for submergence-tolerant varieties such as FR13A. This suggested that *Sub1* reduces a plant's sensitivity to ethylene and therefore reduces elongation and chlorophyll degradation under submerged conditions. This increases

the chance of survival in two ways. First, less energy is wasted on elongation. Second, the plant needs intact chlorophyll to generate more energy during submergence and to resume active growth during recovery from submergence.

More supporting evidence comes from data comparing the performance of Swarna and newly developed Swarna-*Sub1*. Under submerged conditions, Swarna more than doubled its length, elongating by as much as 150%. The submergence-intolerant check variety, IR42, almost tripled its length, elongating by 180%. Swarna-*Sub1*, however, less than doubled in length, with significantly reduced elongation of only 55%. This is comparable with FR13A, which showed 52% elongation.

How did this translate into an ability to survive tolerance? The results were impressive—70% and 80% of, respectively, Swarna-*Sub1* and FR13A plants survived. This compares with a mere 8% of IR42 and 21% of “regular” Swarna plants.

Because of Swarna's short stature, and because *Sub1* further inhibits elongation during flooding, Swarna-*Sub1* is unsuitable in areas prone to flashfloods where water tends to stay in the field at a depth greater than 30 cm. For such conditions, IRRI is developing taller *Sub1* varieties. Swarna-*Sub1* is better suited to areas where floodwater recedes to shallower depths following submergence. 🍌

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*Dr. Vergara is a postdoctoral fellow and Dr. Ismail is a senior plant physiologist in IRRI's Crop and Environmental Sciences Division.*