Resuscitating Rice

Scientists find ways to germinate rice lines kept too long in storage.

Something had gone wrong in the rice plots.

It was supposed to be a fairly routine operation: Seed from 6,000 rice accessions collected from around the world had come to Stuttgart, Arkansas, for planting in field plots to evaluate those rice lines' characteristics. But 211 of the accessions—3.5 percent—never sent up a sprout.

"The 211 that failed to germinate came from 21 different countries," notes Robert H. Dilday, plant geneticist at ARS' National Rice Germplasm Enhancement and Evaluation Center at Stuttgart.

"It's vital that we keep maximum genetic diversity in our rice germplasm collection. Even though some of this rice might not be acceptable from a market standpoint, we never know until we evaluate a line what characteristics it might offer—such as a natural ability to repel weeds—that could be bred into our good commercial rice lines."

The problem with the no-show rice was simply old age, says Dilday. Among the 6,000 accessions seeded, some had been stored as long as 27 years before coming to Stuttgart.

A three-pronged rescue effort was launched to resuscitate the aging seed. First, Dilday and colleague Wengui Yan tried germinating seeds from the nonsprouting lines on moist filter paper in a high-humidity growth chamber.

Twenty-seven lines that had failed in the field produced healthy seedlings in the growth chamber. This technique resuscitated all of the lines

Left: To revive rice seeds that don't sprout naturally, researchers cut out each seed's embryo and endosperm then place them on germination medium in petri dishes.

Right: Those that germinate with this embryo rescue technique are transferred to test tubes filled with growth medium and exposed to 12 hours of light per day.

Facing page: When the seedlings sprout two leaves, they are switched to plastic pots of sterile soil to continue their growth.

Embryo rescue!

that had been stored for 5 years or less and also revived some seeds shelved for up to 19 years.

But 184 lines remained to be revitalized, so Dilday and colleagues next tried culturing the grain on agar medium. Sterilized seeds were transferred to sterile agar medium and left to incubate in a warm, dark site. This revived 58 lines that had typically been stored about 15 years but left 126 in limbo.

"We were just trying bits and pieces of different techniques," Dilday recalls. "Everything we did was a piece of something that had worked somewhere else to some degree, but this was the first application of the techniques in rice."

The researchers had one more roll of the dice: embryo rescue.

First, the seeds were dehulled, sterilized, and set on moistened filter paper in a petri dish for 20 hours. Next, the researchers wielded scalpels to remove each seed's embryo and endosperm, the storage tissue that's used for embryo growth and by the seedling during germination.

Then, they transferred the embryo to a petri dish with germination medium, sealed the dish with paraffin, and tucked the whole package away to incubate in the dark.

As the embryos germinated, they were moved to test tubes of growth medium and exposed to 12 hours of light per day. When the seedlings had sprouted two leaves, they were switched to plastic pots of sterile soil to continue their growth.

Of the 126 remaining lines, 76 pulled through, thanks to the embryo rescue—despite many having been stored more than 19 years.

"These techniques have allowed us to recover rice lines that we thought we'd lost," Dilday says. "We already have some of the best rice in the world here in the United States, but the key to future improvements lies in genetic diversity—being able to tap into characteristics that are naturally present in other rice lines. We can't afford to not preserve this germplasm for future breeding programs."—By **Sandy Miller Hays**, ARS.

Robert H. Dilday is at the USDA-ARS National Rice Germplasm Evaluation and Enhancement Center, Highway 130 East, Stuttgart, AR 72160; phone (501) 673-2661, fax (501) 673-4315. ◆