



Texas Rice

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How Drying Techniques Effect Head Rice Yield

In the United States, rough rice is typically harvested at moisture contents (MCs) ranging from 14% to 24%, and subsequently dried to approximately 12% for safe, long-term storage. Some rice is dried in on-farm, bin drying systems. These systems typically use ambient air, which in Texas, at this time of year, could reach up to 98°F. During the late evening and early morning hours, when the temperatures sometimes drop to 72°F, heaters will kick in to keep the drying temperatures consistent with the daytime highs. However, much of the rice is dried off-farm using high-temperature, cross-flow driers.

creates MC gradients within kernels. Sharma and Kunze (1982) indicate that these MC gradients induce tensile stresses at the kernel surface and compressive stresses at the kernel interior, which can lead to fissure formation within the kernel, and subsequently reduce head rice (HR) yield. To reduce these stresses, tempering is typically practiced, during which kernels are held in a non-drying condition to allow MC gradients within kernels to subside. Intermittent drying/tempering cycles are often used to avoid fissure formation and HRY reductions.

High-temperature drying

When drying rough rice, the glass transition temperature (T_g)



Commercial rice drying operations require intense monitoring of bin temperature, moisture and airflow.

plays a significant role in determining the rate at which moisture can be removed from the kernel. T_g is the temperature at which a state transition occurs, causing the rice kernel to change from a 'glassy' to a 'rubbery' state, or vice versa. Figure 1 shows the inverse relationship between the T_g and MC of a brown rice kernel. For a given MC, if the rice kernel temperature is below T_g , the starch exists in a glassy state; if the kernel temperature is increased above T_g , the starch exists in a rubbery state with much higher diffusivity (rate of moisture transfer), specific volume, and thermal expansion coefficient.

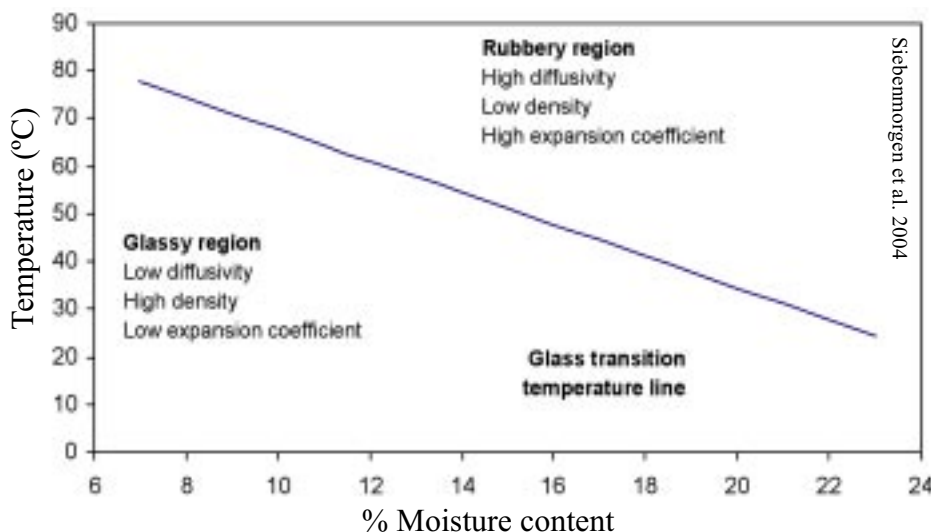


Figure 1. Glass transition temperature relationship for brown rice, indicating the glassy and rubbery regions, as well as the general property trends associated with each region.

From the Editor...



The value of U.S. rice production for 2006 is forecasted at \$1.88 billion, with about half expected to be exported, representing 12% of international rice trade (USDA Newsroom Fact Sheet release no. 0306.06). From these numbers, it is not surprising that the U.S. rice industry underpins many rural communities from California in the West, through Texas, Louisiana, Arkansas, Missouri, and Mississippi in the South. When incorporating a very conservative economic multiplier, the value of U.S. rice to the U.S. economy is at least \$5 billion per year.

Rice prices have been on an upswing of late. Less than two weeks ago, long-grain prices reached ca. \$10/cwt on the September and November futures market, with mills also paying a \$3-4/cwt premium. The increasing prices were good news, and all indications were that they would help to offset the horrendous increases in fuel costs experienced during the recent past.

It is very unfortunate that this upswing market has at least temporarily been disrupted by bad news. On August 18, the U.S. Agriculture Secretary, Mike Johanns, announced that the U.S. long-grain rice market had become contaminated with a genetically modified variety of rice, referred to as LLRICE 601, developed by Bayer CropScience of Monheim, Germany. The transgenes that have been inserted into LLRICE 601 confer resistance to the Liberty Link herbicide. While LLRICE 601 has not been approved for commercial production, the protein produced by the LLRICE 601 transgenes that confers the herbicide tolerance does not affect the quality or safety of the rice grain. Furthermore, the Liberty-linked transgene has been incorporated into two additional rice varieties that have been approved by the USDA for commercial production.

The contamination of commercial rice with LLRICE 601 has not been favorably received by some of the U.S. trading partners. Japan reacted quickly by suspending imports of U.S. long-grain rice. Given that Japan primarily consumes short- and medium-grain rice and very little long-grain rice, the direct impact of

this action on the U.S. long-grain market is small. A far greater concern is whether the contamination will trigger import restrictions from other trading partners. This appears to be the direction things are heading. Dutch officials at the Rotterdam port early today (8/31/06) stopped a shipment out of New Orleans. Given that it has only been two years since the European Union was required by the WTO to end a six-year ban on the import of transgenically modified plant products, and given the higher amount of mistrust in Europe over genetically modified foods, there is concern that this contamination issue could result in further marketing problems.

How serious is this contamination problem? From a human safety perspective, the impact is nil. Numerous tests have failed to find any health impacts. From an economic perspective, however, there will be a cost, even if it is only temporary. This is evidenced by the 5% decrease in the Chicago Board of Trade September and November futures for rice, the two days immediately following the announcement of the contaminated rice. More important, all of the mills in Texas, and possibly the rest of the South, have stopped purchasing long-grain rice. With 80% of U.S. rice production being long-grain, and with an annual value of ca. \$1.5 billion, even a small decrease in price can have a profound impact. Of greater importance is the possible temporary disruption of U.S. exports, such as evidenced by the shipment stoppage at Rotterdam. A critical question is how long will the purchase and shipment of rice be halted? Texas rice producers have begun to harvest their ratoon crop. If sale of the main crop harvest is delayed, this may impact the ability of Texas rice producers to move, dry, store, and sell their second crop as well.

The Dutch action at its Rotterdam port suggests the European Union is now requiring that U.S. long-grain rice be tested for the LLRICE 601 contamina-

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Molecular Markers for Milling Yield

Milling yield, also called “head rice yield”, is the percentage of whole grain obtained from rough rice (paddy rice) after milling. Milling yield is an important trait in rice because it is a major factor determining the price farmers are paid for their crop. To address this issue, the United States Department of Agriculture, Agricultural Research Service Rice Research Unit launched a molecular genetics-based investigation of milling yield, and in October 2003, employed Joe Kepiro as a post-doctoral research associate to conduct the research. The following is a brief summary of the project and the most relevant findings to date.

Breeding for improved milling yield is difficult because the trait has complex inheritance. To determine milling yield, rough (paddy) rice is hulled to produce brown rice (BR). The brown rice is then milled to produce total milled rice (MR), followed by separation into whole and broken kernels. The proportion of whole milled kernels derived from paddy rice is considered head rice (HR), or whole grain milling yield. Each of these milling components is affected by multiple traits (milling sub-components).

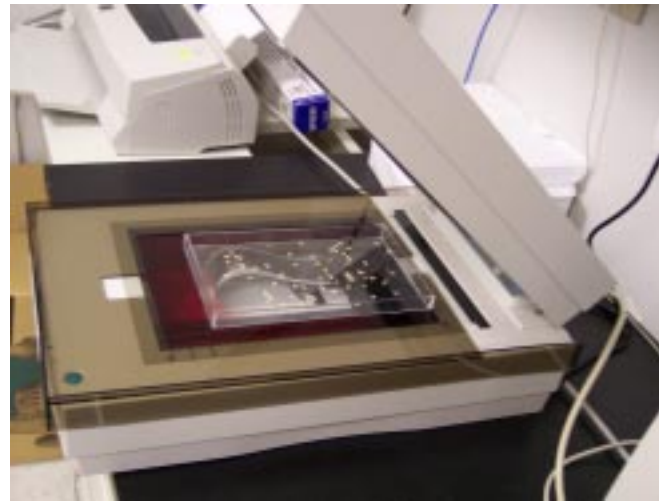


Figure 1. WinSeedle™ Scanner can be used to measure length, width, chalk, green; and determine pre-broken kernels in brown rice.

Many of the sub-component traits are under the control of numerous genes, and therefore the genetic inheritance is complex. Furthermore, these sub-component traits may be affected by environmental (non-genetic) factors, including weather conditions prior to harvest and post-harvest handling of the grain. This makes evaluation and selection of superior milling varieties very difficult and labor intensive.

Identifying molecular markers linked to milling components and their sub-component traits will give breeders new tools to efficiently select varieties with superior milling from large, genetically diverse populations.

This project used 137 advanced progeny lines derived from a Cypress x Panda cross grown in Beaumont during 2002 and 2004 to map gene regions associated with milling yield. Both parents are early maturing long grain varieties. Cypress has intermediate amylose content and is well-known for high and stable milling yield (~ 64%) over a wide range of harvest moisture levels, whereas Panda has low amylose content, and is characterized by low milling yield (~ 52%).

Conventional milling techniques were used to obtain BR, MR, and HR. In addition, the total milled rice recovered from BR (MR/BR), and the proportion of whole milled rice recovered from total milled rice (HR/MR) was calculated. A WinSeedle™ (2005a Pro) color image analysis system (Figure 1) were used to measure kernel lengths and widths of 100 - 150 kernels per family for both brown and milled rice; whereas

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Using Pre-brokens to Select for Milling Yield

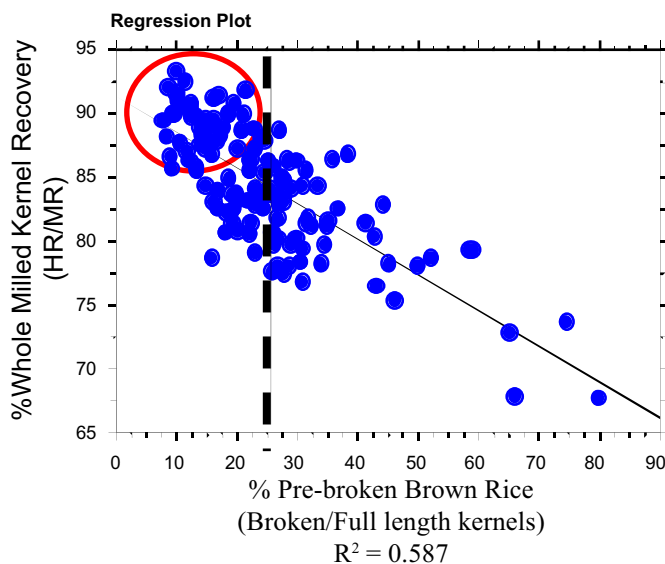


Figure 2. How pre-brokens can be used to streamline selection for higher milling yield: 1) Discard all progeny with values to the right of the dashed line, 2) Mill all progeny to the left of dashed line, 3) Advance progeny in red circle to the next generation. Milling labor can be reduced ~ 50% and only 1/4 of the progeny would be advanced for future testing.

Genetic Markers for Milling Yield continued...

kernel thickness was measured by hand. The number of broken and full length brown rice kernels was used to calculate the proportion of broken in the brown rice after hulling, but prior to milling (pre-broken). The WinSeedle system also allowed development of an accurate method for quantifying the area of chalkiness and greenness on a per kernel basis. Amylose content, which influences cooking quality, was measured using standard procedures.

The important component and sub-component traits of milling yield were identified by statistical analysis. The trait with the largest effect on whole milled rice recovery was pre-brokens (Table 1), and pre-broken is an excellent predictor of milling yield (Figure 2). Interestingly, the traits with the largest effect on pre-brokens were chalk and apparent amylose, not grain dimensions (Table 1). BR was directly correlated to MR, but was unrelated to HR, and MR was moderately correlated to HR. However, chalk and apparent amylose were negatively correlated to both HR and whole milled rice recovery, suggesting starch content and structure impact milling. Length, width and thickness were not associated with HR/MR, indicating that even though the two long grain parents differed in grain shape there was little impact on whole milled kernel recovery.

Significant gene regions were identified for BR, total milled rice recovery, and whole milled rice recovery. One region, qBR-3 on chromosome 3, explained 24% of the variance in brown rice recovery. Region qMR/BR-2 on chromosome 2 explained 14% of the variance in total milled rice recovery (MR/BR). Region qHR/MR-6 (RM190/Waxy locus) on chromosome 6 explained 15% of the variance in whole milled

rice recovery (HR/MR). The region qHR/MR-6 was also the largest contributing gene to the variance in pre-brokens, explaining up to 15.4%. Surprisingly, higher amylose content was associated with lower milling yield in this long grain cross. Significant regions for chalk were identified on chromosomes 1, 2, and 3, and together explained up to 35% variance in chalkiness.

Multiple gene regions for length, width, and thickness were detected, and the percentage of variance explained by the single largest contributing region for these grain parameters was 22.4%, 16.3%, and 21.2%, respectively. The two parents in this cross differed in grain length, and grain shape components, but these traits did not have a major impact on milling in this cross. However, we did identify important regions for grain dimensions that may be useful in breeding programs.

In conclusion, gene regions were identified for the components of milling yield and the most important sub-component traits affecting milling yield in a long grain cross. We determined that pre-brokens is a simple and efficient means of evaluating progeny for milling yield potential. The data for pre-brokens and chalk were measured with a WinSeedle scanner and we encourage breeders to adapt this relatively low cost technology for measuring grain dimensions and chalkiness. We are continuing our investigation to identify additional markers for milling yield in Southern U.S. long grain rice that will help breeders develop varieties with enhanced farmgate value. *

Article by Dr. Joseph Kepiro, email Joseph.Kepiro@ars.usda.gov, Dr. Bob Fjellstrom and Dr. Anna McClung.

Table 1. Correlation of milling components and important sub-components

	BR	MR	MR/BR	HR	HR/MR	PB	Chalk
Milled Rice (MR) - broken and whole kernels	0.82**						
Total Milled Rice Recovery (MR/BR)	0.31**	0.79**					
Head Rice (HR) - whole only	NS	0.38**	0.57**				
Whole Milled Rice Recovery (HR/MR)	-0.20*	NS	0.35**	0.95**			
Pre-broken	NS	-0.20*	-0.41**	-0.78**	-0.77**		
Chalk	NS	NS	-0.27**	-0.49**	-0.48**	0.44**	
Apparent amylose	NS	NS	-0.18*	-0.36**	-0.38**	0.31**	0.33**

* significant at 5% levels, and ** significant at 1% levels.

Drying Techniques continued...

Cnossen and Siebenmorgen (2000) presented a hypothesis incorporating the T_g concept to explain rice kernel fissuring during drying and tempering. To present this hypothesis, Figure 2 shows hypothetical temperature and MC gradients created within a rice kernel during drying. When drying

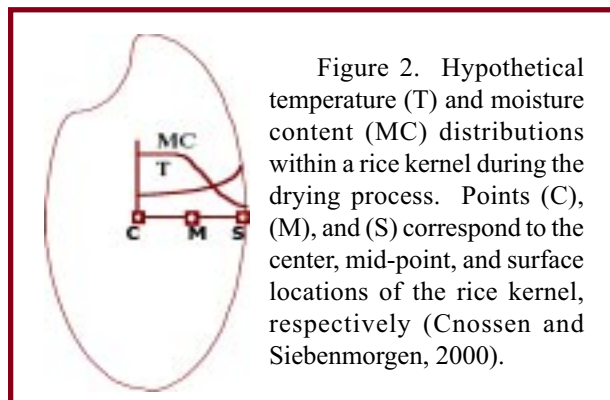


Figure 2. Hypothetical temperature (T) and moisture content (MC) distributions within a rice kernel during the drying process. Points (C), (M), and (S) correspond to the center, mid-point, and surface locations of the rice kernel, respectively (Cnossen and Siebenmorgen, 2000).

with air temperatures above T_g , the rice kernel transitions from a glassy to a rubbery state. As indicated above, this transition dramatically changes kernel material properties, with the thermal volumetric expansion coefficient being of particular relevance. In addition to thermal changes during high-temperature drying, the periphery of the kernel will dry much more quickly than the kernel center, causing an MC gradient within the kernel. Extended drying causes a sufficient volume of the kernel surface to transition to the glassy state (Fig. 3). This creates a situation in which a significant volume of the kernel surface behaves as a glassy material, while the center behaves as a rubbery material. In this situation, the surface volume behaves according to one set of property levels, while the center volume behaves under another, very different set. The T_g hypothesis predicts that if the thermal and hygroscopic property values of the surface and center volumes are sufficiently different in magnitude, and the surface glassy region increases to a sufficient

volume relative to the center, rubbery region, fissures will initiate at the interface of the two volumes.

In addition to the just-mentioned scenario caused by extended drying, the T_g hypothesis predicts that fissures could also be created during post-drying tempering and/or cooling. Depending

on the temperature to which the kernel is exposed immediately after drying (Fig. 4), a sufficient volume of the outer kernel may be forced to transition to the glassy state due to the rapid movement of the cooling front, while the center remains in the rubbery state. As such, if kernels are cooled below T_g before the MC gradient is allowed to subside, fissures will occur due to the surface and center volumes conforming to different properties; this is shown with situation 'B' in Figure 4. Once the MC gradient created by drying subsides, rice kernels can be cooled to temperatures below T_g without incurring fissures.

To save costs, most commercial rice dryers try to reduce MC in as short a period as possible without incurring HR yield reductions. Given the T_g hypothesis, the objective of this study was to determine the maximum MC reduction that could be achieved during the initial drying pass, and the associated tempering durations required, without causing HRY reduction. Drying air temperatures that produce kernel

states both above and below T_g during drying were used. This information is intended to help optimize performance of commercial rice driers.

Two long-grain cultivars, Francis and Wells, at two harvest MCs, were used to determine the maximum MC reduction that could be achieved in the initial drying pass, and the associated tempering durations required, without incurring HR yield reduction. Samples were dried with air at either 60°C/17% relative humidity (RH) or 50°C/28% RH for various durations to create a range of intrakernel MC gradients. Samples were

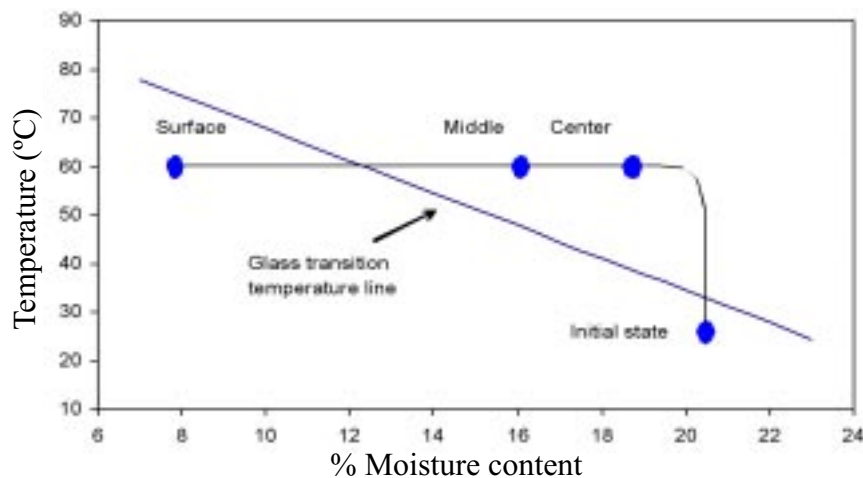


Figure 3. Hypothetical temperature and moisture content gradients within a rice kernel at the locations depicted in figure 2, after extended high-temperature drying.

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Drying Techniques continued...

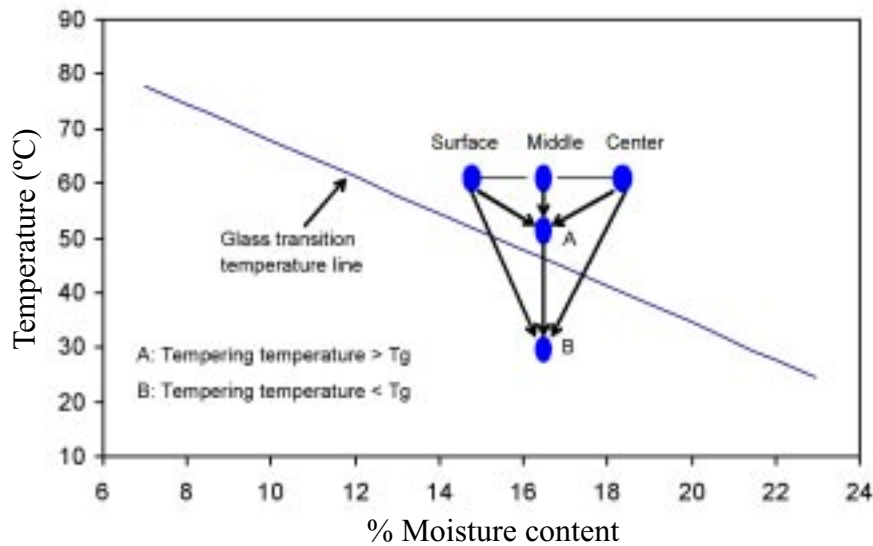


Figure 4. Hypothetical tempering situations above and below the glass transition temperature (T_g) for a rice kernel that had been dried using air temperatures above T_g . Surface, middle, and center correspond to the kernel locations depicted in Fig. 2.

subsequently tempered at the drying air temperature in sealed bags for durations ranging from 0 to 160 min. After tempering, samples were cooled to cause a state transition, and then slowly dried to 12.2% MC. Samples were then milled to determine HR yield. Control samples were dried at 21°C/60% RH.

Results showed that the amount of moisture that could be removed in the initial drying pass was directly related to the harvest MC and the drying air condition. The tempering duration required to prevent HR yield reductions increased with the MC reduction in a drying pass. The HR yield reduction patterns concurred with the hypothesis forwarded by Cnossen and Siebenmorgen (2000) that relates fissure formation during the drying process to rice kernel property changes associated with the glass transition temperature.

The following conclusions were drawn from this study:

- For the drying conditions tested, tempering rice for at least 90 min at the drying air temperature immediately after drying was sufficient to allow intrakernel MC gradients to subside, and thus prevent HR yield reduction during subsequent cooling.

- Plotting the average state points of kernels before and after drying onto a T_g diagram explained whether HR yield reductions would occur due to a drying treatment. Drying air and rough rice harvest MC conditions that placed the average kernel state initially in the glassy region allowed drying rice to a safe storage MC in one pass. However, even for this scenario, tempering was required to prevent slight HR yield reductions. This was presumably due to those high MC kernels within samples that transitioned into a rubbery state during drying.

- For drying air/rough rice HMC conditions that caused a state transition into the rubbery region, the amount of

moisture that could be removed in a single pass without causing HR yield reduction (with sufficient tempering) was directly related to the harvest MC. This MC reduction was dictated by the amount of the kernel surface that transitioned into the glassy state during drying and was correlated to the position of the average kernel state point relative to the T_g line. *

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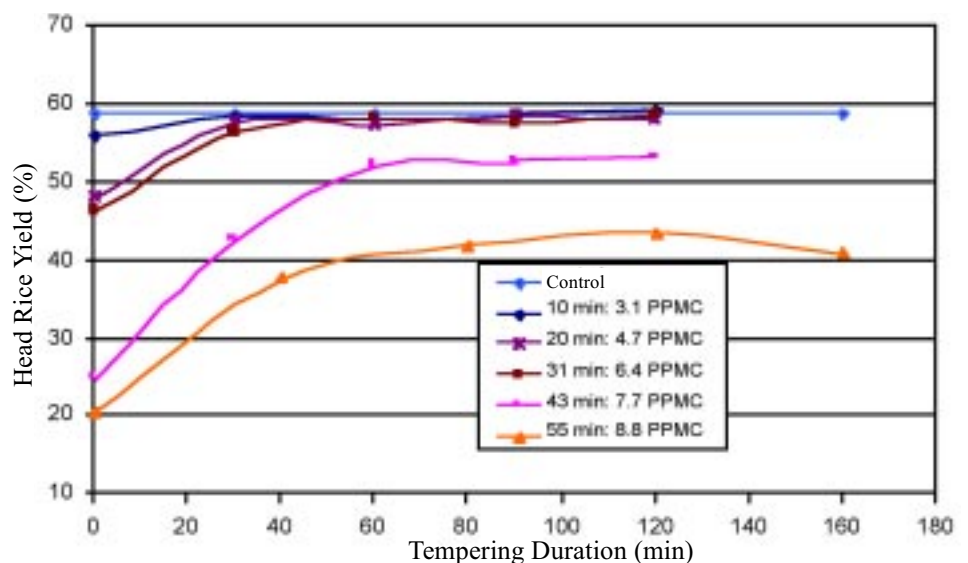


Figure 5. Head rice yield versus tempering duration for cultivar Wells, with a harvest moisture content of 21.6%. Samples were dried using 60°C/17% RH air reducing MC by 3.1, 4.7, 6.4, 7.7, and 8.8 percentage points (MC), respectively, tempered at 60°C for the indicated durations, and then cooled to 21°C.

Moving Science From the Lab to the Classroom

In 1994, the National Science Foundation and the USDA/ARS/SPA funded the Federal Laboratories' Involvement in Research in Science and Technology for Science Teacher Enhancement Program (FIRST STEP). The program was designed to combine the expertise of scientists and science teachers to bring actual research into middle school classrooms.

Through collaboration with Texas A&M's Center for Mathematics and Science (CMSE) the project has evolved into the Future Scientists-Student Outreach Initiative. According to CMSE's, Dr. Craig Wilson, who is the project director, 30 science teachers will go through the program each year. Each year, three USDA/ARS labs serve as the site of a summer institute with 10 science teachers at each site. Last year, Dr. Wilson worked with the USDA/ARS labs in Las Cruces, NM; Stillwater, OK; and Lubbock, TX. This year, he has worked with the labs in Stuttgart, AR; Kerrville, TX; and Beaumont, TX.

This past July, the Texas A&M Agricultural Research and Extension Center at Beaumont, that includes the USDA/ARS Rice Research Unit, hosted Dr. Wilson, along with 10 area science teachers to



The digital microscope on the left displays on the laptop computer. In this photo, the image on the screen is a rice stink bug nymph emerging from the egg case. The teachers that participated in the program were given the microscopes to bring back to their own classrooms.

study the corn earworm. Corn earworms generally, live, eat and breed inside corn husks during a relatively short life cycle, lasting about one month. This makes them a perfect specimen to study in the classroom, as students and teachers can monitor their development from eggs to larvae to pupae to moths.

The teachers learned how to identify the various stages, and determine gender, using a special digital microscope that plugs into a laptop computer. The teachers were given the microscopes to take back to their classrooms so their students could monitor the insects in real-time, take still pictures and even movie clips. The insect specimens are reared at the USDA/ARS Area-wide Pest Management Research Unit in College Station, and come to the teachers in plastic cups with their food supply included. The teachers need only request how many they need for each class and there is no charge to the schools.

During the year, Dr. Wilson visits each participating teacher and teaches in their schools for at least one day to interact with the students. He also makes several presentations about the project at scientific and educational conferences around the nation, such as at the National Science Teachers Conference (NSTA) Annual Conference in Anaheim, CA, which he attended earlier this year, and at the annual conference of the Society for the Advancement of Chicanos and Native Americans in Science (SACNAS) held in Denver, CO.

The project culminates in a Student Presentation Day back at each of the research labs to provide an



Naomi Gipson, Biological Science Lab Technician in the USDA/ARS Rice Quality Lab at the Beaumont Center, participating in the DNA extraction experiment.

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Moving Science continued...

opportunity for student interaction with real scientists. The one at the Beaumont lab is scheduled for May 1, 2007. Each teacher selects five students to represent their science classes. These do not have to be the best students but, rather, those students who have taken a particular interest in the worm research project, or have shown a particular aptitude for science. During the day, each school presents a computer based presentation and/or poster to an audience of teachers, parents and scientists, in an attempt to replicate a scientific presentation or meeting. Then there is a Scientist Q&A Panel made up of four research scientists who volunteer to answer student questions. A catered lunch is provided and interactive tours of research plots and research units take place in the afternoon.

Major funding for the project comes from the USDA/ARS Southern Plains Area, but Texas A&M College of Science provides the infrastructure and salaries for Dr. Wilson and a student worker who rears and dispatches the insects.

According to Timothy Scott, CMSE Director, the initiative targets students in grades 5 – 8 because research has indicated that middle school is the critical point when kids typically lose interest in science. “Today the United States is the breadbasket of the world, but if we don’t rekindle interest in agricultural science in some way, we may not be able to hold on to that heritage,” he adds. “As a nation, we’re facing a critical shortage of agricultural scientists.”

The Beaumont session was specifically timed so that it could be held in conjunction with the Beaumont Center’s 59th Summer Field Tour and was coordinated by Dr. Shannon Pinson, USDA/ARS Research Geneticist. Anna McClung Research Leader for the Rice Research Unit at Beaumont and the Dale Bumpers National Rice Research Center in Stuttgart, AR gave an overview of the Rice Coordinated Agricultural Project (CAP), a multi-state, multi-institution research project that is using genomics to address important problems for the rice industry. Then the teachers were given a tour of the rice quality lab to learn what determines rice quality and were taught a method for measuring starch



On the right, Dr. Craig Wilson, project director of the Future Scientists Student Outreach Initiative with the Texas A&M Center for Mathematics and Science.

content in rice that could be taken back to the classroom. Jillian Lang, Research Associate in the Department of Biological Agricultural Sciences and Pest Management at Colorado State University, led the teachers through a DNA extraction procedure using strawberries and shampoo. Dr. Bob Fjellstrom, USDA Research Geneticist, gave the teachers a tour of the molecular genetics lab, explaining how DNA markers can be used to fingerprint rice cultivars and, finally, Dr. Mo Way, TAES Entomologist, had the teachers wading in rice fields to collect and examine aquatic pests of rice using their new digital microscopes. Some, if not all, of these activities will be carried back to the classroom to spark the imagination of their students.

The focus of the Future Scientists project is on scientific research using the Corn Earworm as a model, but the goal is also to connect resources of local scientific labs to the surrounding educational community. The hope is to inspire a future generation of scientists such as Bernie Rodriguez, a 7th grade student at Cavazos Junior High School, Lubbock, Texas, who participated in the project last year and said. “You have inspired me to want to become a scientist when I grow up. I will remember every single experiment. It’s not often that someone comes to our school and we do fun experiments.”

For more information visit the website at <http://futurescientists.tamu.edu> *

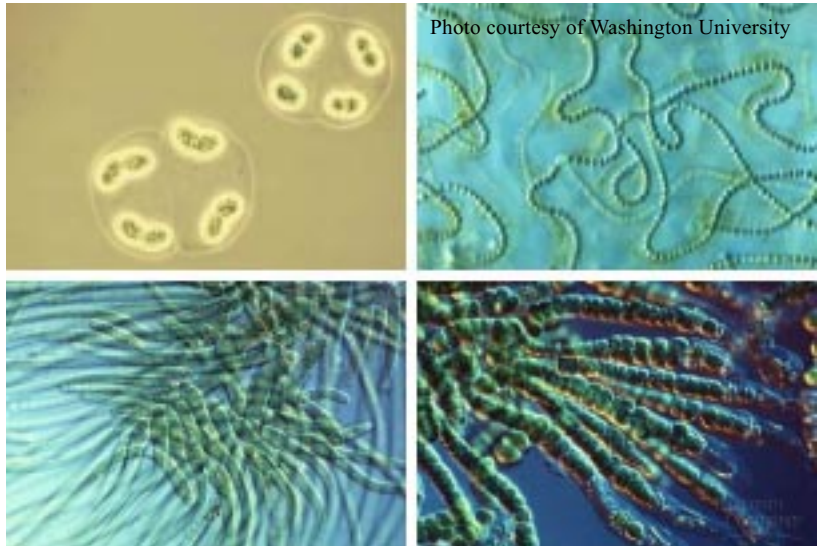
Cyanobacteria: A Potential Nitrogen Source in Rice Fields

Cyanobacteria are a group of prokaryotes that are autotrophic, from the Greek words *auto*, which means 'self' and *tropho*, which means 'nourishment'. They were once classified as blue-green algae because of their superficial resemblance to eukaryotic green algae, in that they are able to capture energy from the sun and turn it into glucose through the process of photosynthesis.

Cyanobacteria have the distinction of being the oldest known fossils, with specimens found dating back 3.5 million years. Many scientists believe that the cyanobacteria were tremendously important in shaping the course of evolution and ecological change throughout the earth's history. The oxygen atmosphere that we depend on was generated by numerous cyanobacteria during the Archaean and Proterozoic Eras. Before that time, the atmosphere had a very different chemistry, unsuitable for life as we know it today. The other great contribution of cyanobacteria is the origin of plants. It is generally accepted in the scientific community that the chloroplast with which plants make food for themselves is actually a cyanobacterium living within the plants cells. Based on the fossil record, scientists hypothesize that sometime in the later Proterozoic, or in the early Cambrian, cyanobacteria began to take up residence within certain eukaryotic cells, making food for the host in return for a safe home. This event is known as endosymbiosis, and is believed to be the origin of eukaryotic mitochondria.

Cyanobacteria get their name from the bluish pigment phycocyanin, and they also contain chlorophyll a, the same photosynthetic pigment that higher plants use. A third pigment, phycoerythrin, imparts a red or pink color. The Red Sea gets its name from occasional blooms of a reddish species of *Oscillatoria*, and African flamingos get their pink color from eating *Spirulina*. These pigments are embedded in specialized tissues called lamellae, which are the analogues of eukaryotic thylakoid membranes. The lamellae are found in the normal vegetative photosynthetic cells that all cyanobacteria possess.

Some species have the ability to differentiate into



Single cell and filamentous cyanobacteria, the latter being most commonly found in flooded rice fields.

other cell types. One is a climate resistant spore called akinetes, which form when environmental conditions become harsh. They can persist in the soil for long periods until favorable conditions return.

A third cell type, known as heterocysts, are of particular interest for agricultural purposes, as this is where nitrogen from the air is transformed into compounds that plants can utilize. Nitrification cannot occur in the presence of oxygen, so these cells have an especially thickened wall that creates an anaerobic environment, and houses the enzyme nitrogenase that makes nitrogen fixation possible. Using this enzyme, these species of cyanobacteria are capable of fixing atmospheric nitrogen (N_2), and converting it into forms that plants can use, like ammonia (NH_4), nitrites (NO_2), and nitrates (NO_3).

Although they are truly prokaryotic, cyanobacteria have an elaborate and highly organized system of internal membranes in addition to those already discussed. They widely distributed organisms, and must possess a high potential of adaptation to diverse environmental factors. For example, it is well documented that UV-B radiation can negatively effect processes such as growth, pigmentation, motility, as well as the enzymes of nitrogen metabolism. Many cyanobacteria have developed a number of adaptive strategies to reduce the negative effects of excessive radiation including the synthesis of UV screening

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Cyanobacteria continued...

pigments, and the production of chemical scavengers that detoxify the highly reactive oxidants that cause damage. Screening pigments include scytonemin and mycosporine-like amino acids. Cyanobacteria such as *Scytonema* and *Nostoc*, two of the more common species found in rice paddies, form filaments embedded in a mucilaginous sheath, which contain these screening compounds. These organisms are more tolerant of UV-B radiation than those that do not contain such covering.

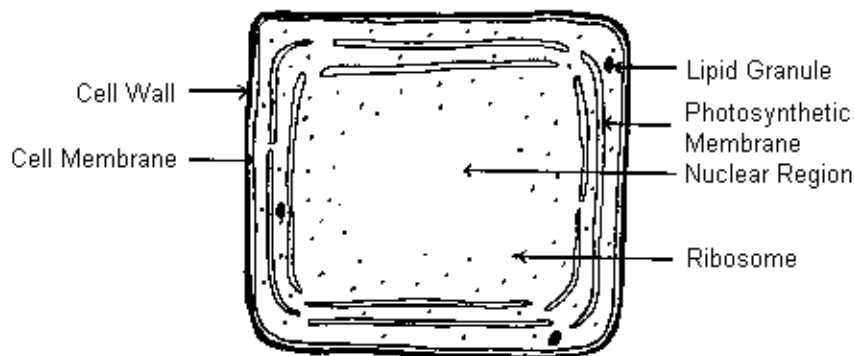
Other species common in rice paddies include *Calothrix*, *Fischerella* and *Anabaena*. In India, Egypt, the Philippines and many of the Eastern rice producing countries, *Anabaena* is used extensively in rice production through its symbiotic affiliation with the floating water fern, *Azolla*. However, in the United States, *Azolla* is on the Federal Noxious Weed List, so its use is highly discouraged, and inter- as well as intra-state transport is regulated by the federal government.

Still, the other filamentous species just mentioned have great potential for use in commercial rice production, and a large body of scientific evidence exists that shows the contribution of nitrogen can be significant.

In a paper published in the Australian Journal of Plant Physiology, scientists at Bangladesh Agricultural University reported noteworthy yield increases using cyanobacteria inoculants in rice production. Results of their field trials showed that cyanobacteria biofertilizer helped reclaim problem soils, such as acid and saline soils, improve their fertility status, and had the potential to supplement 25 – 35% of the nitrogen needed for production.

In this study, there were 8 fertilizer treatments: control (nothing added), recommended fertilizer dose (RFD), RFD – 15%N, RFD – 15% N +cyanobacteria (CB), RFD – 30%N, RFD – 30%N + CB, RDF – 45% N, RDF – 45% N + CB.

Five agro-ecological zones covering major regions of Bangladesh having five distinct soil types were selected for the study. The soil types were acid, calcareous, saline, red and neutral. The researchers evaluated the soil samples and chose the three most predominant and productive strains of cyanobacteria



at each location. These were reared in the laboratory for inoculation at the field test sites. The maximum cyanobacterial population was found in calcareous soil, and the least in the acid soil, with the ranking as follows: calcareous>neutral>saline>red>acid.

Considering this ranking, it's not surprising that for yield and nitrogen uptake, the most pronounced increases were in the acid, red and saline soil. In fact, in the acid soil, the treatment that reduced nitrogen by 30%, but added the cyanobacteria, the yields were actually higher than the plots where the full recommended fertilizer dose was given.

Regarding the pH of the different soil types, application of the cyanobacteria raised the pH of the acid and red soils, and lowered the pH of the calcareous soil, bringing all of them closer to neutral.

There is a growing body of evidence, from this study, and from research conducted in Australia, that cyanobacteria biofertilizers drastically improve the soil fertility status and reduce the salinity of soil. Australian scientists are working with farmers on the islands affected by the 2004 Indian Ocean tsunami to reduce the salinity of their soils using cyanobacteria.

Another benefit of these biofertilizers is that they produce bioactive substances that promote plant growth. According to research conducted at the Universidad de Buenos Aires, these substances include plant growth regulators, such as auxins and gibberellins, as well as amino acids and vitamins. In this study rice seedlings were treated with different strains of cyanobacteria. While the response varied, all the strains tested caused significant more growth than the controls that did not receive the treatment.

Although there is much work to be done to maximize the benefits for rice farmers, it is clear that this is an avenue worth exploring. *

From the Editor continued...

tion prior to shipping, and requiring the shipped grain be certified as not being contaminated with LLRICE 601. My understanding is that contamination will be defined as having greater than a 0.1% threshold level of contamination. The difficulty with this stance is that the only way to insure that shipped rice is below a contamination threshold would be to conduct extensive testing on each shipment. Practically speaking, to detect say 0.01% contamination, with a threshold of 0.1% contamination, with 95% confidence, would require that 475 grains be randomly sampled from each shipment (see the equation listed below to calculate sample size). This is obviously very difficult, but not impossible to do. The key is making sure the grains are selected from different parts of the shipment.

The disruption of long-grain shipments will affect both the U.S. rice industry, and rice consumers in the E.U. The 25 E.U. countries would be faced with an impossible task of finding alternative import markets for long-grain rice. There are only a few countries that export high quality long-grain rice to Europe. A sufficiently high price might entice China to sell some of their short supplies, but with China's rice breeding programs jumping into the transgenic rice game big time, this suggests that there might be a high chance of contamination with transgenic rice. Vietnam and Thailand are large long-grain rice exporters, but the elasticity in their supply is limited, at least in the short-term. A few South American countries have historically exported long-grain rice, but given recent droughts in that continent, this is an unlikely source. The fact-of-the-matter, there is very little flexibility in finding replacement export markets for long-grain rice in the short-term.

The scope of the contamination is not known. As a starting point for determining the extent of the contamination, Bayer has requested that each of the public and private rice plant breeding programs test their foundation seed. Bayer has developed a test to determine the presence of the herbicide tolerant transgene that was originally placed in rice by Bayer. The test should be in place by the time this issue of *Texas Rice* is published. Foundation seed comes directly from each of the rice plant breeding programs, and is the first step in the production of commercial rice seed for sale to rice producers. However, looking at it a bit differently, Foundation Seed is also the last step in a very

long and time consuming process that each and every rice variety must go through prior to the release of a new variety.

If contamination by LLRICE 601 is found in any of the seven long-grain U.S. rice breeding programs (five state programs, one USDA program, and one private rice breeding program), the cost required to "clean-up" the contamination will be considerable. Each of the U.S. rice breeding programs evaluates around 20,000 to 30,000 rice lines each and every year. Each of these lines is created by manually collecting the pollen from the male part of flowers from one or more rice plants from a variety having desirable traits, and manually inserting the pollen into several individual flowers from a different rice variety having another set of desirable traits, from which the male parts had been removed.

Most rice breeding programs produce up to around 250 crosses each year, with a total of a few dozen seeds produced for each cross. As you can imagine, this process is extremely tedious, very costly, and must be done one flower at a time. Once a "cross" between two rice varieties is made, the seeds that are produced are collected, isolated, planted the following year, then repeatedly evaluated over a several year period to eliminate undesirable types. From the original 20,000 to 30,000 lines evaluated each year, all but a few will be discarded as not having a sufficient number of the desired traits. Anywhere from 7-12 years is required for a single rice genotype to go through this gauntlet. Not surprising, this means that each new variety takes several years and several \$100,000 to several \$ million to develop. Lots of questions remain in the event one or more of the U.S. rice breeding programs have been contaminated.

From another perspective, given the lack of any health risks with this herbicide tolerant rice, if Bayer can quickly work with the USDA to deregulate LLRice 601, and work quickly with the E.U. to put in place acceptable testing, the problem could quickly disappear. A possibly more important question revolves around whether USDA policies on the testing of transgenic plant material can be changed to further minimize this type of problem. It is not clear how this would be done, without seriously slowing future plant-based biotechnological advances. How would additional

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regulations on testing prohibit the illegal “catching” of transgenic seed? How would additional regulations prevent acts of biotechnology sabotage? And, how would additional regulations prevent, with 100% certainty, the exceedingly rare movement of transgenes by insect pollinators or by wind? At some point, all of the regulations in the world will not prevent with 100% certainty the willful and illegal movement of restricted material. This points the finger back to the need to allow shipments below an accepted threshold level of contamination to be approved for export.

Were we dealing with a transgenic product that threatens human health, there obviously would be valid concerns over contamination and transport. However, in the case of LLRice 601, and the transgene that it contains, this is absolutely not an issue of a human health concern. If additional regulations involving the growth of transgenic plant material were suggested by regulatory agencies, one would hope there would be provisions put in place to distinguish between different levels of danger, from none in the case of the Liberty Link trait, to possibly a higher danger classification for genes that could wreak havoc on society were they to become broadly established in the environment. Let's hope common sense prevails.

As society changes, challenges such as this one will occur from time to time. On a positive note, transgenics will continue to play a very valuable and increasing role in our society. From insulin production, to crops having greater protection from insect pests, to nutraceutical crops, to unique microbes that can degrade toxin chemicals or convert plants into biofuels, transgenics is here to stay. Let's hope this unfortunate issue of contamination is quickly solved

and behind us by the next issue of *Texas Rice*.

For anyone who might be interested, the following equation can be used to estimate the number of grain that would need to be sampled to determine if a contamination is below or above an acceptable contamination threshold.

$$n = t^2 |p - T|^{-2} p q \quad (\text{From Wilson 1985})$$

where:

n = the number of grain that would need to be randomly selected for sampling

t = Student's t and has a value of 1.96 for a 95% confidence interval estimate

p = the contamination level as a proportion (the original contamination discovered in Arkansas and Missouri has a 0.0006 p value, or 0.06%)

T = the acceptable contamination threshold expressed as a proportion of grain that are contaminated (I understanding the threshold for the European Union is 0.001, or 0.1%)

P = the proportion of grain that are not contaminated (the proportion of grain in the contaminated Arkansas and Missouri samples that were not contaminated was estimated to be 0.9994 or 99.94%)

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Sincerely,



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