

The Rice Foundation

Research Project Report Form

Please use this form to clearly and concisely report on project progress. The information included should reflect quantifiable results that can be used to evaluate and measure project success. Technical reports, no longer than 4 pages, may be attached to this summary report.

Project # and Title	13-21 New Knowledge and Improved Methods to Increase Breeders' Ability to Develop Rice Varieties with Enhanced Fissure Resistance.
Investigator & Organization	Dr. Shannon Pinson and Dr. Yulin Jia- USDA-ARS; Dr. James Gibbons, UARK
Reporting Period	Final Report – research conducted over 3 years, 7/2006 – 4/2009

I. Statement of the project objective(s).

Develop new knowledge on the genetic inheritance of rice kernel fissure resistance and new selection techniques that can be used by breeders to develop fissure resistant rice varieties.

To be accomplished by **a)** determining if genetically combining multiple mechanisms of fissure resistance can further increase resistance levels by evaluating fissure resistance in recombinant inbred lines derived from Cybonnet x Saber, and **b)** measuring the efficacy of early generation selection for fissure resistance by evaluating F3 and F4 progeny trait shift resulting from selections applied in the F2 and F3 generations of a Cypress x LaGrue cross.

II. Statement of quantifiable progress toward project objective(s) achieved during this reporting period.

A. Documented, for the first time ever, successful early-generation selections for FR using in-lab fissure inductions (see Figure 1). While the in-lab evaluation method remains laborious for widespread breeder use, there is excitement among breeders (i.e. Drs. Tabien and Gibbons) over using it to speed the recovery of progeny with good milling quality from wide crosses, such as those involving foreign parents of poor milling quality.

B. Tentatively found that a FR gene resides on chromosome 1 – detected through its linkage with the sd1 gene. Details are in text of Figure 1. This corroborates equally tentative results from the parallel, federally funded RiceCAP study.

C. At the initiation of this project, we knew that Cypress' fissure resistance (FR) was largely attributed to its hull, knew that Cybonnet had inherited its FR from Cypress, and we suspected that Saber had a different FR mechanism, possibly attributed to rapid moisture diffusion through its endosperm. ***This project provided new data supporting the existence of two different FR mechanisms in the fact that some Cybonnet/Saber RIL progeny were even more FR than Cypress, Cybonnet, and Saber,*** while other progeny were highly susceptible.

D. Improved breeding germplasm: the highly FR Cybonnet/Saber progeny will be evaluated further to see if they warrant release as a variety. At the least, these selections will serve as improved breeding parents for introducing both the Cypress and Saber FR mechanisms

Achievements continued next page

E. Developed two key gene-mapping populations. The FR versus FS Cypress/LaGrue progeny identified through the early-generation selections can now be evaluated for unequal marker distributions which indicates linkage with the FR genes we successfully selected for. Association with *sdl* was already detected, suggesting that a FR gene resides on chromosome 1. The Cybonnet/Saber RILs have now been characterized for FR over 2 locations, 1 year. A second year of FR data, plus marker characterizations is all that is required to map FR genes in this newly created population that will be able to reveal Saber as well as Cypress FR genes.

F. The populations can also serve as novel research tools for identifying grain traits underlying FR – such as verifying that reduced hull diffusivity is indeed segregating with FR among the Cypress/LaGrue progeny, or that high endosperm diffusivity segregates with FR among the Cybonnet/Saber RILs.

III. Summary.

For the first time ever, and to the excitement of several rice breeders, we documented successful selection for FR among early breeding progeny (F2s) based on resistance to in-lab fissuring. The lab method remains too laborious for widespread breeder use. Identification of molecular gene-tags linked with FR genes to enable marker-assisted selection of FR by breeders is suggested.

Data supporting the hypothesis that Cypress and Saber have two different FR mechanisms was collected.

Two populations suitable for mapping of FR genes from both Cypress and Saber, and for studying the grain traits that are responsible for FR were created, facilitating continued research.

IV. Publications relating to this research (please indicate pending).

Pinson, S.R.M., J.W. Gibbons, and Y. Jia. 2009. Effective Early-Generation Selection for Rice Resistant to Kernel Fissuring to Hasten Breeding Efforts. Rice Utilization Workshop, "Beneath The Hull: Exploring the Health-Promoting Functions of Rice Starch and Protein." New Orleans LA March 12-13, 2009.

Pinson, S.R.M., J.W. Gibbons, and Y. Jia. 2008. Early generation selection for resistance to rice kernel fissuring proves effective. Agronomy Abstracts paper number 551-7, Houston, TX Oct 5 - 9, 2008.

Pinson, S.R.M., J.W. Gibbons, and Y. Jia. Early generation selection for resistance to rice kernel fissuring was accomplished with a laboratory selection method. *Being drafted for submission Crop Science*.

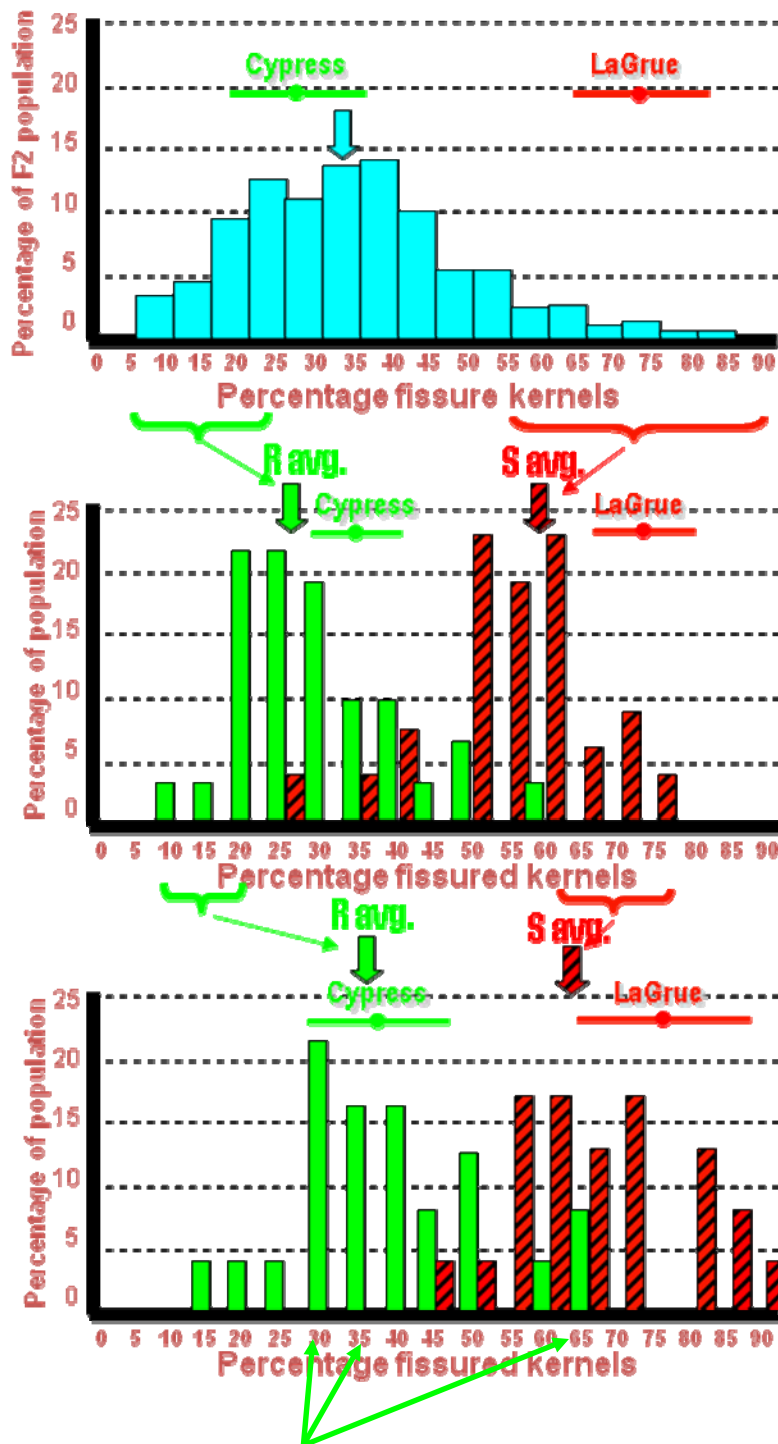
V. Message, questions, comments or requests.

ATTACHMENTS:

1. Figure 1 = summary of breeding progress accomplished through F2 and subsequent F3 selections, complete with F4 progeny testing.
2. Abstract of exciting F2-selection progress presented orally at the American Society of Agronomy meetings, Oct. 5-9, 2008, Houston, TX. (note, presentation was made prior to completion of the grain analysis for F4 progeny testing)
2. Poster of results as presented to the Rice Utilization Workshop, March 12-13, 2009, New Orleans, LA. A similar poster was also presented to producers and industry personnel at the Beaumont Rice Field Day, July 2008. (note, presentation was made prior to completion of the grain analysis for F4 progeny testing)

Please e-mail this completed form to Sharon Guy at sguy@usarice.com. If you have any questions, please call Chuck Wilson at 870-673-7541.

Figure 1. **The F2 selections proved successful** in that the F3 progeny of the FR F2 plants were, on average, significantly more FR than the progeny of the FS F2s. Bold arrows indicate the averages of the progenies of the FR and FS selections.. The lines under Cypress and LaGrue in the figures represent the average (dot) +/- 1 sd.



F2 Selections in 2006:

We applied 10% selection pressure in 2006, selecting 30 FR and 30 FS F2 progeny from among 300 Cybonnet/Saber RILs. The skewing of the F2s toward FR suggests that FR may be dominant over FS.

F3 Progeny testing in 2007

The FR F2:3 progeny were, on average more FR than the FS-selected F2:3s.

A second round of selection was applied in 2007, during which an association between FR and *sd1* was noted. All of the most- FR F3 selections were semidwarf, while most but not all of the FS selections were tall, suggesting that a FR gene may be near *sd1* on chromosome 1.

F3:4 Progeny testing in 2008

showed shorter tails, and less overlap between the divergently selected progeny than seen in 2007, which may indicate that the second round of selection made additional breeding progress, though may also be a year-effect.

LaGrue was in the susceptible tail each year, suggesting that compared

2008 data supported linkage between FR and *sd1*, and dominance of FR over FS:

Three sibling F4 lines descendant from the same FR F3 family appeared to segregate for FR in 2008. Marker data revealed that the FS sibling was homozygous *Sd1*, while the two FR siblings were homozygous for *sd1*, further suggesting linkage between *sd1* and a FR gene. This also lends support to the hypothesis that, at this *sd1*-linked FR gene anyway, FR is dominant to FS.

ATTACHMENT 1

Abstract of oral presentation made at the American Society of Agronomy Meetings, October 5-9, 2008, Houston, TX.

Early Generation Selection for Resistance to Rice Kernel Fissuring Proves Effective.

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Rice milling yield refers to the proportion of kernels remaining unbroken after mechanical removal of the hull and bran layers. Whole kernels have two to three times more market value than broken kernels. Any reduction in milling yield will result in direct profit losses for both producers and millers. Exposure of the rice kernels to severe moisture changes before or after harvest can cause them to fissure, and is one of the leading causes of reduced milling yield. 'Cypress', a southern U.S.A. rice cultivar released in 1993, is known for its resistance to kernel fissuring. This cultivar is not widely grown today, having been replaced with cultivars having higher yield potential and disease resistance. While breeders would like to incorporate Cypress' fissure resistance into improved cultivars, their efforts are limited due to a lack of methods for identifying and selecting for fissure-resistance in early breeding generations. A laboratory method wherein small samples of seed are evaluated for fissure rates after controlled rewetting has proven to reliably identify fissure resistance among pure-breeding material grown in several replicated environments. This study investigated how effective the technique is as an F₂ selection tool. Seed from 300 unreplicated Cypress/LaGrue F₂ plants was evaluated for resistance to induced fissuring. The 10% most resistant (R), and 10% most susceptible (S) progeny were selected for planting into F₃ panicle rows in 2007. F₃'s were planted in two locations (TX and AR), two replications per location. Fissure resistance measured on seed harvested from five individual plants per F₃ row showed significant differences between the mean and distribution of the R and S populations. The average fissuring rate among the R selections was 20%, while the S selections averaged 35% fissuring. The efficacy of selection in the F₃ generation is the focus of further study.

Effective Early-Generation Selection for Rice Resistant to Kernel Fissuring to Hasten Breeding Efforts



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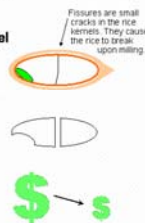
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PROBLEM

LOSSES from Rice Kernel Fissuring

- Broken rice has **lower market value** than whole milled kernels (ranges from 1/2 to 3/4 the value).
- Because fissured rice tends to break upon milling, rice kernel **fissuring directly reduces income** for producers and millers.



What Causes Kernel Fissuring?

When **moisture** from rain, dew, or humid air re-enters a dry(ing) rice kernel, the outer starch layers swell, creating inward **pressure** that can cause stress fractures, or cracks, in the rice endosperm. San et al., 1979. J. Ag. Eng. Research 72: 247-251



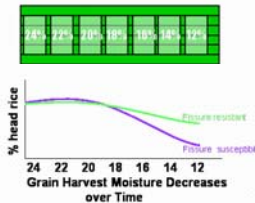
How can rice RESIST fissuring?

HULL: the hull can keep moisture out, like a sealed plastic bag. The fissure resistance exhibited by the variety **Cypress** appears to be largely hull-related. (Haggoner et al., 2003, Am. Soc. Ag. Eng. paper no. 036193)

ENDOSPERM: stressful swelling of the outer layers can be prevented by rapidly diffusing the moisture through the endosperm. (Haggoner et al., 2003, Am. Soc. Ag. Eng. paper no. 036193)

Saber is fissure resistant even though it re-wets rapidly, so it is hypothesized that its resistance is due to diffusion of moisture through its endosperm.

High Amounts of Seed and Labor Required to Evaluate Resistance to Field Fissuring Limited Breeding Progress



The most widely accepted method for evaluating resistance to field fissuring involves **sequential harvests** over time of sequentially more-mature (drier) grain samples from the same research plot. Up to 15 grain samples per plot are harvested, threshed, dried and milled to evaluate milling quality of that variety over time. Varieties that are susceptible to field fissuring show a rapid loss in milling quality over time, while resistant varieties show a slower loss in milling quality.

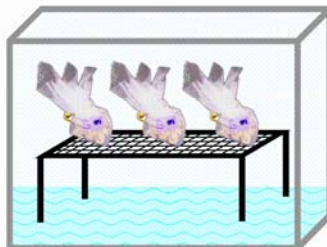
Because such research requires large plots, large seed supply, and much labor, breeders are not able to 'select for' fissure resistance in early breeding generations. Instead, breeders are left hoping that they have not thrown out fissure resistant progeny during the 5 to 7 generations of selection for other traits they pursue while reducing lines and increasing seed supply to allow fissure evaluations.

SOLUTION

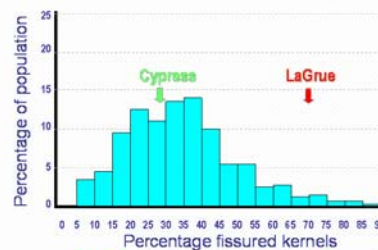
New Laboratory Evaluation Method Proves Successful at Selecting for Fissure Resistance in the 1st Breeding Generation based on merely 50 rice kernels.

Selection for fissure resistance among segregating F₂ (first breeding generation) plants was recently accomplished.

Selection was based on percentage fissured kernels after exposing small 50-kernel samples to controlled levels of fissure-inducing humidity under controlled laboratory conditions.

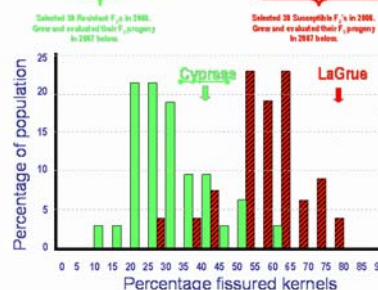


Water in the bottom of a sealed plastic box provides 99% humidity when held at 46 °C (115 °F). This allows us to expose the enclosed seed samples to a controlled level of fissure-inducing conditions.



Seed harvested in 2006 from 300 **Cypress** x **LaGrue** F₂ plants ranged from 6 to 87 % fissured after the controlled humidity treatment. **Cypress** resists fissuring, and averaged 27% fissured. **LaGrue** is susceptible to fissuring, and averaged 70% fissured kernels.

F₂ progeny from 30 **Resistant** and 30 **Susceptible** F₂ plants were grown in 2007.



The average fissuring rate among the F₂ families selected as **Resistant** was significantly less than the fissuring within the **Susceptible** F₂ progeny.

IMPACT: Breeders' efforts to develop new rice varieties improved for their fissure resistance will be more rapid and more successful using this newly proven laboratory selection technique.