A major objective of the RiceCAP effort is to conduct educational workshops on the biotechnology of rice. As part of this effort, Drs. Jim Oard and Rick Nelson organized the third RiceCAP workshop entitled “The RiceCAP DNA Marker Workshop: Markers, Mapping, and Beyond” held at the Noble Foundation, Ardmore OK, June 4-10, 2006. A total of 26 participants from 10 U.S. institutions attended the workshop. Topics for the workshop included a wet laboratory over a two day period that emphasized hands-on high through put DNA isolation methods, troubleshooting, useful tips, and traps to avoid. Extraction protocols were provided for different tissue types. Robotic sample extraction method was demonstrated. Other procedures included DNA template quantification, set-up and overnight run of PCR, genotyping of parents and lines of an actual RiceCAP mapping population, and software analysis of marker data generated by the participants.

Over a period of five days, a total of nine lectures and computer laboratory exercises were provided by eight invited speakers. The first speaker was Jim Oard from the LSU Agricultural Center who gave an overview and introduction to DNA marker technology. Clare Nelson from Kansas State University then reviewed theoretical and practical aspects of constructing linkage maps in plants. Rebecca Doerge from Purdue University nicely summarized principals and applications for QTL mapping, permutation thresholds and “e-QTLS” from microarray studies. In a second lecture, Clare Nelson established fundamental concepts for basic data relations for markers, insights to marker and PCR product analysis. Luca Comai from UC Davis lectured on the TILLING procedure for functional genetic polymorphisms and gene silencing. Guo-Liang Wang from Ohio State reviewed principles and applications of SAGE and MPSS technologies. Finally, Dave Kudrna from University of Arizona provided an overview of the

(Continued on page 2)
2006 DNA Marker Workshop (cont.)

excursion on Lake Murray. A big thanks is extended to Rick Nelson and all the staff at the Noble Foundation for making this workshop a great success!

People

From the Dale Bumpers College of Agricultural, Food and Life Sciences Alumni Magazine:

Don McCaskill, BSA ‘70 MS ’73, received the 2006 Outstanding Alumnus Award at the Honors Day Convocation on April 20.

He joined Riceland Foods in Stuttgart as Manager of Rice Research and Development in 1981, became Director of Research and Development with technical responsibility for all Riceland products in 1996 and was named Vice President for Research in 2001.

He has had a major impact on enhancing the value of Arkansas crops by further processing while also creating jobs and expanding payrolls in rural Arkansas. He was instrumental in development of a patented rice parboiling process; the creation of new rice-based consumer, foodservice and food ingredient products; and development of markets for rice bran oil.

As a University of Arkansas student, Don McCaskill developed leadership skills that have served him well. He was president of the Agricultural Students Association and received the ASA Senior Leadership Award. He was inducted into Alpha Zeta, Gamma Sigma Delta and Omicron Delta Kappa honor societies and was named Outstanding Senior in Alpha Gamma Rho fraternity.

He helped organize the Bumpers College Alumni Society as a charter member of the Board of Directors. He has been a member of the Institute of Food Science and Engineering Advisory Board since its inception, was a member of the Food Science External Review Team in 2003 and has served on the Agricultural Development Council. He has long served on the board of directors of the Ozark Food Processors Association and has been president and vice president twice.

For the rest of the article, please go on-line at: http://www.uark.edu/depts/agripub/Publications/Graduate/grad.sprsum06.pdf
Louisiana producers planted 170,000 fewer acres of rice this spring, a decrease of almost one-third from last year’s 530,000-acre crop, according to the USDA’s June 30 Acreage Report.

Rice specialists said the 32-percent decline to 360,000 acres was mostly due to salt levels left on the soil by Hurricane Rita, the storm that struck southwest Louisiana and east Texas one month after Hurricane Katrina devastated Louisiana and Mississippi.

But last year’s low prices and continuing economic problems also took their toll. “We know of some fairly prominent farmers who have just gotten out of the business,” said John Saichuk, Extension rice specialist with the LSU AgCenter. “They couldn’t face another year with the problems we’ve had.”

Delta Farm Press, 6/30/06

For the full article, please see: http://deltafarmpress.com/news/060630-USDA-Acreage-Report/
Year 2 RiceCAP Technical Summary of Overall Project Progress

A broad goal of the RiceCAP project is to bridge an ever widening gap between molecular biology efforts on rice research and applied aspects of rice breeding to develop high value rice cultivars for commercial production in the U.S. The RiceCAP project includes applied and basic rice scientists from 15 different institutions from 13 states. Although communication continues to be a challenge among such a diverse group of scientists, meetings continue to be scheduled to maximize the cooperative efforts of both the applied field scientists and the more molecular biology oriented laboratory scientists. The current report reflects the cooperative effort of all of the RiceCAP participants.

The RiceCAP project was initiated in two phases with the management phase of the project started in September of 2004 and the scientific phase started in January 2005. December of 2005 marked the completion of the first full year of the scientific effort on RiceCAP. Thus, the Continuation Award being presented here includes an update on the project progress over the past 18 months (January 2005 through June 2006). The Plans of Work for Year 3 of the Project cover September 2006-August 2007.

There have been several notable changes in the second year of the RiceCAP effort. Some minor modifications have been made to the composition of the advisory boards to specifically provide additional expertise in the milling yield component of the RiceCAP effort. In addition, five new projects (with 9 new PI’s) that focus on the various RiceCAP objectives were added in year 2. The projects selected for funding out of the flexible funds component of the budget were selected based on scientific merit and relevancy to the RiceCAP objectives. The proposals were evaluated by the RiceCAP Executive Committee, the Scientific Advisory Board, and the Stakeholder Advisory Board. Following a similar evaluation process, proposals will be solicited from the RiceCAP PI’s in September of 2006 for bottleneck efforts that will support efforts of immediate importance to accomplishing the RiceCAP goals in year 3.

A meeting on the first year of progress of the project was held in February 2006 in conjunction with the Rice Technical Workers Group, to fully assess progress, bottlenecks, and future directions based on input from the Stakeholder and Scientific Advisory Boards. Based on recommendations from the Scientific Advisory Board, in consultation with the Executive Committee, two projects were not supported for additional funding to focus more of the resources on more immediate priorities.

An active website (http://www.uark.edu/ua/ricecap/) continues to provide information about the project including project goals, research progress, participating laboratories, past and upcoming events, and outreach efforts.

A significant level of progress has been made in the first 18 months of the RiceCAP effort which includes developing the foundation rice mapping populations for the effort to improve milling yield and sheath blight resistance, the evaluation of microsatellite molecular markers to evaluate the segregation of these complex traits, QTL analysis to identify genomic areas of interest associated with these traits, the functional analysis of specific candidate genes directly controlling complex traits, particularly sheath blight resistance, conducting training workshops on molecular marker assisted breeding and biotechnology to evaluate gene function (VIGs), and the development of informational and instructional posters, presentations, and modular displays for various target audiences.

A summary of the RiceCAP effort by objective is included below:

Objective #1: Identify and use candidate genes and other molecular markers linked to quantitative trait loci which control milling quality and resistance to sheath blight disease.

(Continued on page 5)
Technical Summary (cont.)

(Continued from page 4)

MY1 RT0034/Cypress Mapping Population

Some 500 F11 random lines from the MY1 population were provided to the RiceCAP project by RiceTec, Inc., Inc., Alvin, TX. The MY1 population was reduced to 156 lines by scrutinizing the progeny with markers and elimination of those with nonparental alleles or alleles from only one parent. These were genotyped with 155 SSR markers and the data used to initiate QTL mapping. Based upon molecular marker analysis, 156 F12 lines were selected for planting during 2005 at Beaumont, Crowley, and Stuttgart. Data were collected throughout the season on some 133 agronomic traits. Samples from all locations were shipped to Stuttgart for cleaning and then to Beaumont for milling to avoid any differences in processing methods at the different locations. At Beaumont, brown spot disease symptoms were severe and milling yield of the parents and other commercial checks in the study was observed to be very low (ranged 29-46%). Thus, the Beaumont location was dropped from the milling analysis but was used to identify QTL associated with brown spot resistance.

At the two remaining locations, Cypress proved to have higher milling yield, lower pre-brokens (broken grain that occurred in the field prior to milling), lower grain chalk, shorter grain length, and broader grain width than RT0034. However, the two parents were similar for the percent of green (immature) kernels. Although the Crowley location had higher percentage of green, chalk, and pre-broken kernels, the frequency of the population was shifted to higher milling yield than at Stuttgart. The results demonstrate that grain dimension traits are quite stable over locations however other factors influencing milling yield are sensitive to the environment.

Harvesting the families at optimum grain moisture is a subjective assessment based upon the change in color of the panicle associated with ripening of the grain. An analysis of harvest moisture and grain moisture at milling demonstrated that a large number of the samples at one location were subject to overdrying in the field or after harvest. Not suprisingly, the most important single factor affecting whole grain milling yield was % pre-brokens, the proportion of grain cracking that takes place in the field prior to milling. These post-harvest parameters may have pre-disposed the grain samples to cracking in the field and being more susceptible to induced fissuring which may explain the lack of correlation in whole milling yield between the two locations ($r = 0.098$). The next steps for analysis of the phenotypic and genotypic data will include covariate analysis, principal component regression, discriminant analysis, and a mixed models approach for the QTL analysis. However, at this stage, factors associated with grain ripening and post-harvest handling (% pre-brokens, moisture at milling) appear to have the largest impact on whole milling yield. Other traits, hypothesized to have a significant impact (heading, tillering, grain dimension, etc) on whole milling yield, were not very influential.

A preliminary QTL analysis of the MY1 population was conducted by simple interval mapping (SIM) for the ~50 directly measured traits (most replicated twice in two or three environments (AR, LA, and TX) and with a composite interval mapping (CIM) analysis of key milling traits. CIM is a well-accepted method analogous to multiple regression for constructing a genetic model to explain phenotypic observations. Although a full QTL analysis of MY1 has not been completed, there were no plausible QTLs for milling yield (let alone QTLs with increasing alleles from Cypress, the superior-milling parent) and mostly marginal or submarginal QTLs from components such as breakage or fissuring. There were obvious QTLs for highly heritable traits such as amylose content and days to heading, not of direct interest for milling yield. A full mixed-model CIM analysis has been developed that accommodates locations and replicates within location and returns tests for QTL, location, and QTL x location effects. It can also handle multiyear data. This model, now implemented in MATLAB but to be ported to Java for speed, will be used to complete the analysis of MY1 and will be applied to forthcoming RiceCAP MY and SB data sets. Full analysis of a QTL dataset should take into account the correlations among the traits analyzed, the variation among environments and replicates within environments, possible QTL x QTL effects, possible pleiotropy, domain knowledge about the

(Continued on page 6)
control and expression of the traits including parental phenotypes (which suggest the expected source of superior QTL alleles) and an assessment of the significance threshold for declaration of a QTL, done by permutation testing or other method.

The disappointing initial findings, the allele skewing, and the reduced size of the MY1 population led to a general decision to drop it from further phenotyping in 2006 and proceed with more detailed QTL analysis before collecting any additional trait data. Not identifying QTL for milling yield may be due, in part, to the segregation distortion observed in this population. Allele segregation at a marker locus in a RI population is expected to be 1:1, with a small number of heterozygotes. The number of marker genotypes homozygous for RT0034 was thus expected to equal that for Cypress. In fact the proportion was 12298 : 6603, with 646 heterozygotes and 446 missing. The most extreme segregation in favor of RT0034 was 114:10:5 at a marker on chromosome 11. Only two markers favored Cypress, with the most extreme segregating 59:66. Segregation distortion toward indica alleles has been seen in other indica x japonica crosses, e.g. Moroberekan x CO39 (reported in 1993 by G.-L. Wang and colleagues). Furthermore, a large number of the original population was discarded because they were believed to be selves because of the high proportion of RT0034 alleles when screened with about 10 markers. It is possible that screening some of these families with more markers may demonstrate that they were truly segregating offspring and not selves. Thus, if phenotyping MY1 in 2007 is warranted to collect another year of data, it would be possible that the number of families in the population could be increased if some of the original discarded lines were reclamed. The initial QTL analysis identified a few significant QTL for sub-components of milling yield (i.e. chalk, pale green kernels, etc) and MY1 families possibly segregating for these putative QTL were planted at Beaumont for future QTL validation. If other QTL are identified through additional analyses, MY1 families possessing these can be grown in winter nurseries or in 2007 summer nursery for verification.

Affymetrix GeneChip Rice microarrays are now commercially available and could potentially make high-throughput detection or scoring allelic variation possible if differential hybridization of genomic DNAs from different rice lines could reveal polymorphism. The objectives were to test the ability of a gene chip strategy to identify and score single feature polymorphisms (SFPs) in the parents of our mapping populations.

DNA from the parents of the MY1 and MY2 populations were hybridized to Affymetrix chips and hundreds of potential polymorphisms were observed as differences in hybridization intensity. Roughly 1000 were estimated MY2 and roughly 5000 were estimated in the MY1 population. Putative SFPs were verified by sequencing the corresponding genomic fragments from both parents and the hybridization results were found to be reasonably reliable.

Although the results from this work looked very promising, the group also found good levels of polymorphism using microsatellite markers. The Array-based SFP markers may be useful when fine mapping QTL however, this part of the project has been put ‘on hold’ at least temporarily so that resources can be used to complete the QTL mapping of all of the selected populations. Once QTL have been verified this technology can be used to generate very high density of markers for fine mapping.

MY2 Cypress/LaGrue Mapping Population

Approximately 500 F4 lines of the MY2 population were harvested in 2004 and planted in the winter nurseries. F5 panicles were harvested and planted in the 2005 breeding nurseries in Beaumont and Crowley. However, the materials in Beaumont were lost as a result of hurricane Rita. Although the parents of the MY2 population (Cypress and LaGrue) differed by only three days in heading like the MY1 parents, the progeny of this mapping population were observed to range in heading by just 14 days (as compared to 30 days for MY1). This suggests that the MY2
population will have fewer confounding factors that may impact milling yield than the MY1. The F5 progeny are currently under marker evaluation. Although a large number of outcrosses were identified and discarded from the MY1 population, an initial screen of some 300 progeny of the MY2 population with five markers indicates that only 1% of the progeny are outcrosses. A total of 230 polymorphic markers between the parents of MY2 population indicating that there is good marker saturation for identifying QTL associated with milling yield in this population.

A total of 325 families were planted in summer 2006 at Crowley and Stuttgart in replicated trials. A comparison of the MY2 parents demonstrated a wide difference in whole milling yield when grown at Crowley, but little difference when grown at Stuttgart. LaGrue grown at Crowley also had higher %chalk, % green, and high susceptibility to induced fissuring - factors associated with lowering milling yield. In Stuttgart, many of the milling components were similar between the two parents. Moreover, Lagrue (considered the low milling parent) had lower susceptibility to fissuring as compared to Cypress (high milling parent) at this location. Pre-brokens appeared to be an important factor associated with milling yield in MY1, and was a differentiating factor between the MY2 parents at Crowley.

DNA was isolated from the 325 progeny from the Cypress/Lagrue in preparation for genotyping during 2006. Approximately 150 robust markers giving good map coverage were selected from the 230 polymorphic markers identified in the MY2 parents.

**MY3 L204/01Y110 Mapping Population**

The development of the MY3 cross is proceeding under the direction of Dr. Farman Jodari at Biggs, CA. Both summer and winter nurseries are being used to advance the population which includes 312 RILs at this time. The population will be advanced by selfing and F5 progeny will be ready for phenotypic and genotypic analysis during Year 3.

**SB1 Rosemont/Pecos Mapping Population**

Dr. Arun Sharma was responsible for conducting the genotypic analysis of the SB 1 population that had been previously phenotyped. A total of 143 SSR markers were mapped in 279 F3 families in the population, completing genotyping efforts in SB1. Interval mapping analysis identified four QTLs (LOD ≥ 3.5) for SB resistance on chromosomes 1, 2, 3 and 9. The QTL on chromosomes 2 and 9 and possibly 3 were independent of effects due to height or maturity. Families segregating for these QTL were planted in Beaumont to produce progeny that can be used for confirmation of QTL and possible fine mapping.

**SB2 Cocodrie/ MCR 010277 Mapping Population**

The SB2 double haploid (DH) population has been registered and released to the public as a mapping population. It was evaluated in inoculated sheath blight nurseries during 2005 and was observed to vary from 3-8 in disease ratings (on a scale of 0-9). Seed produced from the 325 families was distributed for planting at three locations in 2006 (Stuttgart, Crowley, and Beaumont). In addition, it will be screened using the micro-chamber and mist chamber methods during 2006.

Nine SSR markers were examined on each of the 325 lines. No nonparental alleles were observed as well as very little heterozygosity and haplotypic redundancy, indicating there are few problems with the way the population was generated.

The SAB suggested that the SB2 population should be considered for milling analysis. Although not enough seed was available of this population for use in a milling study during 2006, the parents of SB2 were included as repeated checks in the 2006 MY2 field study as well as the MY1 and MY2 parents. This will allow an assessment of the divergence between the SB2 parents for milling parameters and to compare with that of the milling populations parents. If milling evaluation of SB2 is warranted, seed will be produced in 2006 for planting in 2007.
Technical Summary (cont.)

(Continued from page 7)

SB3 Cocodrie/ Chu 0066601

A total of 280 DH lines were developed for SB3 and advanced through the winter nursery by Dr. Chu. The population was evaluated for uniformity and reaction to sheath blight disease at Crowley in 2005. Because of the higher level of resistance found in MCR 010277 (SB2) as compared to Chu 0066601 (SB3), and its excellent adaptation (i.e. its resistance is not due to late maturity or tall plant height), the SB3 population has been put on hold and resources will be used on the other populations.

SB4 Lemont/TeQing TIL Mapping Population

Approximately 120 TILs were identified for further field evaluation for resistance to infection by the sheath blight pathogen. From this study, 93 lines were identified for marker evaluation to identify TILs having the putative SB resistant QTLs that can be used for fine-mapping. Molecular marker analysis was conducted to identify TILs to be used for fine mapping experiments and determine if lines possessing single QTL can be identified as being more resistant than the susceptible parent, Lemont. Selected TILs were backcrossed to Lemont to make populations segregating for single sheath blight QTL. Four QTL regions were selected for targeting for fine mapping efforts.

RILs of the Lemont X Jasmine 85 population are being developed by single seed descent. Jasmine 85 has relatively strong resistance compared to other germplasm but is considered undesirable by breeders because of other traits. The goal of this effort is to obtain 200 F8 RILs for fine mapping candidate genes identified by DNA Microarray and SAGE projects described in objective 2.

New efforts under Objective 1 initiated in the past year include a project to evaluate sheath blight resistance in wild rice species, a project to help establish MAS programs in Missouri and Mississippi and a project to develop a standardized method to purify and quantify RS-toxin from cultures of Rhizoctonia solani in an effort to develop an accurate means of assessing genetic susceptibility to the toxin. Such a method may be useful for verifying putative QTL for sheath blight resistance that provide only moderate levels of resistance when isolated from other QTL.

Objective #2: Validate the function of candidate genes associated with sheath blight resistance and milling quality

Candidate genes are being identified based on the QTL and association mapping (see Objective 1), gene expression profiling, signal pathway analysis, protein interaction mapping as well as literature and database searches. About 40 candidate genes have been selected for functional validation via transgene overexpression, RNA interference and mutant analysis (if T-DNA or transposon mutants are available). Transgenic analysis for sheath blight resistance and milling quality will be conducted in collaboration with the PIs in Objective 1.

Due to the complexity involved in the phenotyping and mapping of sheath blight resistance and milling yield QTL loci, good candidate genes are not yet available for functional validation. Therefore, we have selected about 40 candidate genes based on differential expression, protein-protein interaction and/or previous QTL analysis. These genes encode downstream defense components which may mediate broad-spectrum resistance against rice pathogens including Rhizoctonia solani. A wide range of gene constructs have been made. Rice transformation in Nipponbare and Kitaake is being conducted for functional validation of genes via RNA interference, and/or transgene overexpression. To accelerate the functional analysis of defense-related genes the virus-induced gene silencing method was validated using the Spl11 E3 ligase gene in the Nelson lab. More of an effort will be made to utilize U.S. germplasm in the candidate gene experi-

(Continued on page 9)
Technical Summary (cont.)

(Continued from page 8)

ments in the coming year.

Preliminary data from Leach lab showed that silencing of oxalate oxidase genes resulted in increased susceptibility to sheath blight, blast and Russian wheat aphid, suggesting a positive role of oxalate oxidase in conferring broad spectrum resistance. Preliminary analyses in Yang lab of transgenic lines defective in defense signaling suggest that ethylene and OsMPK5 signaling may play a significant role in sheath blight resistance. In addition, many other transgenic lines (e.g., NH1) from Ronald and Wang labs are being analyzed for sheath blight resistance.

Progress Report (Feb. to April 2005)

Two Robust Long-SAGE (RL-SAGE) libraries have been constructed using RNAs isolated from control and R. solani-inoculated Jasmine 85 leaves. A total of 11,448 and 11,317 distinct significant tags (>2 copies) were identified from the control and the infected libraries, respectively. About 36% of the tags were specifically present in the infected library and 55% were shared between the two libraries. Many novel genes related to defense and metabolism were highly expressed in the infected library. To identify candidate genes related to seed development/milling yield, three MPSS libraries have been made from RNAs isolated from developing seeds of three rice cultivars (Cypress, LaGrue and Nipponbare) at 6 days after anthesis. Over 1.0 million signatures were obtained from each library. Clustering analysis resulted in 13,000 to 19,000 distinct transcripts in the libraries. The matching rate to the Nipponbare genome sequence ranged from 85% to 88%.

Genes whose expression are induced or repressed following sheath blight inoculation may be candidate genes underlying resistance QTL. Changes in gene expression has been examined using Agilent microarrays. Approximately 200 genes that appeared up-regulated at 16 hours after infection were sent to Clare Nelson for in silico mapping.

Objective #3: Develop technical training programs and resources to ensure the implementation of molecular markers and gene validation technologies to solve rice problems.

Four well-attended training workshops also have been conducted by RiceCAP in the past two years including “Molecular Markers – Unleashed” at the , “The Application of Virus Induced Gene Silencing”, “The Use of Gramene”, and “Markers, Mapping, and Beyond”. The workshops have been actively attended by RiceCAP participants, as well as traditional plant breeders, students, and technical personnel. Discussions are underway to conduct two additional workshops in year 3 of the project with the topics predicated on surveys from past workshop attendees.

Objective #4: To effectively communicate the science and potential of rice plant genomics, including progress and description of the RiceCAP, to the U.S. rice industry

The Outreach component of RiceCAP has developed a number of tools to help communicate the use of biotechnology to improve economically important traits in rice. The tools have included talks, posters, logos, pens, and post-its to draw attention to the overall project goals, objectives, the website, and the personnel involved. The information has been presented to over 2,000 people at various rice field days and meetings and the information presented has been widely covered by the popular press. In addition, the Outreach group has been involved with specific workshops with grade school children and teachers communicating the techniques and value of biotechnology. These teaching activities have included enthusiastic participation by both the students and the teachers. Furthermore, the teaching activities have involved a large number of under represented students. Future efforts will continue to communicate the RiceCAP goals to a broader audience and conduct surveys to assess the effectiveness of the communication tools being utilized.

The complete RiceCAP Continuation Award Proposal for Year 3 can be found on-line at: http://www.uark.edu/ua/ricecap/RiceCAP_Continuation_Award_2006final.pdf.
## Calendar of Events

### July 2006

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### Schedule of Events

- **7/11/06**—The Southwest Louisiana Rice Tour, The Vermilion Parish LSU AgCenter Rice and Model Farmer Field Day, Klon-dike, LA, Main RiceTec Field Day, Alvin, TX
- **7/13/06**—Beaumont Rice Field Day
- **7/20/06**—Delta Research and Extension Center Field Day
- **8/3/06**—SEREC Crops Field Day, Robwer, AR
- **8/9/06**—RREC Field Day, Stuttgart, AR
- **8/16/06**—Cache River Valley Seed Field Day, Cash, AR
- **8/23/06**—The Missouri Rice Farm Field Day, Glennonville, MO
- **8/30/06**—California Rice Field Day, Biggs, CA
- **8/31/06**—The Delta Center Field Day, Portageville, MO

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**RiceCAP**

A coordinated research, education, and extension project for the application of genomic discoveries to improve rice in the United States. A project supported by the National Research Initiative (NRI) of the Cooperative State Research, Education and Extension Service (CSREES).

We’re on the web!  
www.uark.edu/ua/RiceCAP