

*New Strategy IDs Deleted Genomic Regions in Rice Mutants*

Myron Bruce, Colorado State University

Detection of genomic deletions in rice using oligonucleotide microarrays is a powerful tool to study genes involved in complex traits.

In a paper recently published in *BMC Genomics* (2009, 10:129; doi:10.1186/1471-2164-10-129), Myron Bruce, a PhD student in Jan Leach's lab at Colorado State University, and their collaborators demonstrated that a microarray-based approach could be used to rapidly and accurately identify deleted genomic regions in rice mutants.

Mutants are critical tools for genetic approaches to tease apart biochemical and metabolic pathways, and to determine gene function in plants. Over the past few years, researchers have used various strate-

gies to develop insertion mutant collections for rice. These types of mutations, which can result in disruption or activation of gene expression, are very useful because the mutation is 'tagged' by the element used for insertion. However, the generation of these mutants has generally been limited to *japonica* rice varieties which are more amenable to transformation and regeneration than *indica* varieties. This is unfortunate, as *indica* varieties represent the predominant rice type grown in the world (~80%) and harbor many interesting traits important for rice production.

Another approach to generating mutants is to induce genomic deletions by treatment with chemical and irradiation mutagens. This strategy provides a rapid and relatively inexpensive method to obtain large pools of mutants. Furthermore, any genotype can be used because there is no need for transformation, and the density of mutations generated allows for genome-wide saturation with relatively small

populations.

In rice, a collection of over 40,000 mutants induced by various chemical and irradiation strategies was developed in the *indica* rice cultivar IR64 by Hei Leung and colleagues at the International Rice Research Institute (IRRI). IR64 was chosen because it is the most widely grown *indica* rice in Southeast Asia and because it contains a large number of valuable agronomic characteristics. The variety of mutagens was selected to ensure a collection with a range of deletion sizes, providing the opportunity to identify a mutation in a single gene or a deleted group of genes that might collectively govern a trait (e.g., quantitative trait loci, QTL).

While the collection contains many mutants with interesting phenotypes, identifying what genes are mutated is difficult because the mutations are not tagged (no marker from an insertion event). Thus, time and labor intensive mapping strategies were

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### RICE FACTS & FOLK-LORE

To plow 1 ha of rice land in the traditional way, a farmer and his water buffalo must walk 80 km.

The average person in Myanmar eats 195 kg of rice each year; in Lao PDR and Cambodia, it's about 160 kg. Contrast this with the average European, who consumes 3 kg per year and the average American, who eats 7 kg.

Source:

<http://www.asiarice.org/sections/learnrice/ricefacts.html>



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## China and GMO Rice

Wang Feng, a rice researcher at the Fujian Academy of Agricultural Sciences, and his colleagues have been developing genetically modified (GM) rice strains to resist pest infestation, and have been testing in these plots for a decade. Two strains from Wang's team are now awaiting regulatory approval by the agricultural ministry for commercial growth. It could represent the largest commercialization of a GM food-crop. Rice is a staple for most of the country's 1.3 billion people and a primary source of calories for more than half the world's population.

China's population is set to top 1.45 billion by 2020, and the country needs to increase grain production by about 25%, a daunting task in the face of increasing urbanization, industrialization, farm-

land reduction and the efflux of rural workers to the cities. The Chinese government has latched on to transgenic plants as a solution, rolling out a major research and development initiative on GM crops for the next 12 years, including a sizeable investment of 25 billion yuan (US\$3.7 billion) from the central government and additional matched funding from its provincial counterparts. China is the world's largest rice producer, weighing in with nearly 200 million tonnes, and several observers fear that introducing GM rice could endanger the food supply and the environment. "The consequences would be unthinkable if large-scale cultivation of GM rice were not properly regulated," says Xue Dayuan, chief scientist on biodiversity at the Nan-

jing Institute of Environmental Sciences. But in a country where policies are rarely a matter of open debate, government officials warn that the scale of the impending food shortage makes further delays an unaffordable luxury. "This is the only way to meet the growing food demand in China," says Huang Dafang, former director of the Biotechnology Research Institute of the Chinese Academy of Agricultural Sciences (CAAS) in Beijing.

Wang is optimistic that his group's pest-resistant GM rice will help lead the way. Kong Luen Heong, an entomologist at the International Rice Research Institute in Los Baños, the Philippines, calls pest-resistant GM crops a short-term fix for long-

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## Workshops and Conferences

### 5TH IRBC WILL BE HELD IN LITTLE ROCK, ARKANSAS (USA), IN 2010

The 5th International Rice Blast Conference will be held in Little Rock AR at The Peabody Hotel, August 11–15, 2010.

The conference schedule is still in the planning phase and details/links are being made available as plans are finalized on the conference web site <http://www.ars.usda.gov/Main/docs.htm?docid=18212>.

Conference check-in will be Wednesday, Aug 11, 2010. Presentations will be two days, Aug 12-13. Field tours to the Stuttgart AR area and the Dale Bumpers National Rice Research Center will be conducted on Saturday, Aug 14. A banquet will be held Saturday evening at The William J. Clinton Presidential Center.

On-line conference registration will be made available at a later date as arrangements are implemented.

The chairman of the local organizing committee is Dr. Yulin Jia, [yulin.jia@ars.usda.gov](mailto:yulin.jia@ars.usda.gov).

### Bacterial Disease Research

A group of three researchers from Iowa State University and one from Kansas State University are looking at two bacterial diseases of rice. The team is studying bacterial blight of rice, which is caused by a bacterium called *Xanthomonas oryzae pathovar oryzae*, and bacterial leaf streak of rice caused by the closely related bacterium *Xanthomonas oryzae pathovar oryzicola*. The group is researching why some types of rice are naturally resistant to the bacteria and how the bacteria change gene expression in susceptible rice plants.

Full article at [http://esciencenews.com/articles/2009/04/01/research-ers\\_examine\\_bacterial\\_rice\\_diseases\\_search\\_genetic\\_solutions](http://esciencenews.com/articles/2009/04/01/research-ers_examine_bacterial_rice_diseases_search_genetic_solutions)



## People

### FEI G. RUBINELLI JOINS JIA'S LAB AT DBNRRC

Dr. Fei G. Rubinelli received her B.S. from Shandong Agricultural University with emphasis on understanding of the molecular basis of bacterial wilt disease on potato. Her M.S. is from Kansas State University where she worked with Dr. Pfender researching biological control of *Cephalosporium* stripe disease on wheat. Her Ph.D. research was conducted with Dr. P. Wong at Kansas State University on protein isolation and characterization of

glutamine synthesis. Her postdoctoral training was with Dr. Marzluf at Ohio State University and focused on transcriptional regulation in *Neurospora crassa*.

Since March 1, 2009, Dr. Rubinelli has been assisting various molecular projects on rice and pathogen relations including rice with *Rhizoctonia solani* in Dr. Yulin Jia's laboratory at USDA-ARS Dale Bumpers National Rice Research Center in Stuttgart, Arkansas.



Dr. Fei G. Rubinelli, DBNRRC.

### STEFANO COSTANZO JOINS BROOKS' LAB AT DBNRRC

Dr. Stefano Costanzo is a post-doc Research Plant Pathologist with the USDA DBNRRC in the Molecular Genetics Lab with Dr. Steven Brooks. Costanzo joined Brooks' lab in April after two years research with a rice blast project funded by the USDA Headquarter and supervised by Dr. Yulin Jia in the Molecular Plant Pathology Lab, DBNRRC. In the Molecular Genetics lab, he will be mapping a gene that controls sensitivity to a phytotoxin produced by the rice sheath blight fungus *Rhizoctonia solani*. This project is an extension of Brooks' efforts with Rice-CAP. Costanzo's project will be the first map-based cloning effort for a gene in-

involved in sheath blight resistance.

Costanzo has had research experiences on many economically important crops such as tomato, potato and soybean as well as very destructive pathogens such as *Phytophthora infestans*, *P. sojae* and *P. ramorum*. He received his Ph.D. in Plant Pathology from The Pennsylvania State University. His dissertation focused on the characterization and mapping of QTLs (Quantitative Trait Loci) conferring resistance to late blight disease in potato. As part of his graduate research, he developed, based on RFLP markers, a complete chromosomal linkage map of a diploid potato population.



Dr. Stefano Costanzo, DBNRRC.

Costanzo's expertises are gene mapping, cloning; molecular markers development and gene expression studies. His most recent research involved the discovery of 12 distinct alternative splicing events in a major rice blast resistance gene.

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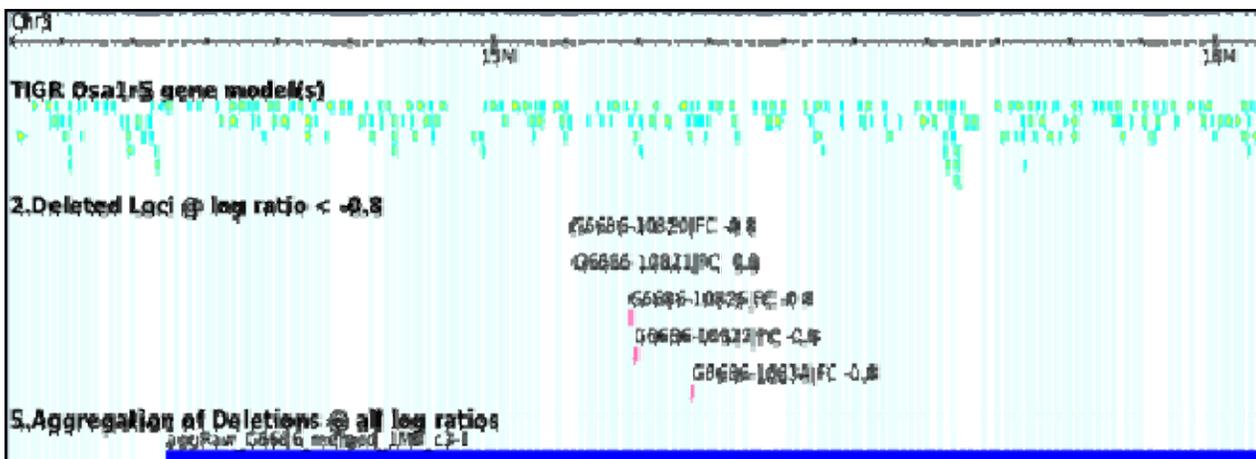
needed to identify the mutated genes. The advances in genomic tools presented an opportunity to overcome this problem, and expedite the process for identifying the mutated genes or genic regions.

For the discovery process, Bruce hybridized genomic DNA from mutants to the Affymetrix Rice GeneChip® that contains more than 55,000 probe sets representing 48,564 gene models ([http://www.affymetrix.com/support/technical/datasheets/rice\\_datasheet.pdf](http://www.affymetrix.com/support/technical/datasheets/rice_datasheet.pdf)). In proof-of-concept studies with known deletion mutants, the conditions for hybridizations and the parameters for analy-

sis to discover the deletions were established. Additionally, the authors demonstrated the ability to reliably predict genomic deletions without replicating array hybridizations, greatly reducing the costs usually associated with array research. The process was then applied to a larger set of mutants where the deletions were unknown. The deletions detected in the rice mutants ranged in size from one gene model to ~500 kb and deletions were found on all 12 rice chromosomes. By this approach, the researchers were able to rapidly identify candidate genes responsible for a phenotype of interest. Overall, the study demonstrates not only the utility of microarray-based method for forward genetics, but also its potential as a reverse genetics tool through

accumulation of hybridization data for a collection of deletion mutants harboring multiple genetic lesions. Ramil Mauleon (IRRI) collaborated with the group to develop a user-friendly genome-browser that shows the positions of mutations aligned onto the rice pseudomolecules and allows for rapid identification of untagged mutations [http://irfgc.irri.org/cgi-bin/gbrowse/IR64\\_deletion\\_mutants/](http://irfgc.irri.org/cgi-bin/gbrowse/IR64_deletion_mutants/).

**How can this information further the RiceCAP initiative?** Bruce's study shows that it is feasible to develop a database of characterized mutants with deletions that span regions of interest in the genome. His data suggests that there is a 91% probability of detecting a deletion in each gene at least once using



Screenshot of the user-friendly genome browser developed by Mauleon (IRRI) in collaboration with Bruce's research. The positions of deletion mutations are aligned onto the rice pseudomolecules to allow for rapid identification of untagged mutations. The browser allows for selection of different log ratio cutoffs, providing flexibility in data analysis. Preservation of the information in a genome browser on all deletions in the same lines will provide important information for researchers investigating the functions of other genes.

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only 3,000 mutants generated by Fast Neutron or Gamma Ray treatments, a reasonable number of mutants to handle. As more and more mutants are hybridized onto the arrays, the resource will be a valuable tool for researchers to identify deleted regions

that have been associated with QTL. Identifying these deletions will allow analysis of the contribution of genes to these complex phenotypes, such as sheath blight resistance.

Myron Bruce was interviewed by BioArray News to get a better sense of how his could impact the broader

agbio community. The interview can be found at : <http://www.genomeweb.com/arrays/qa-colorado-state-scientists-size-affy-attempt-build-rice-mutation-database?emc=el&m=368571&l=9&v=f9ac429a6c>.



Leaves from mutant plants used in the study showing the distinctive *spl1* lesion mimic phenotype (F1856, G650, F2045 and G9799) compared to the wild type parent (IR64). Prior to the study, F1856 and G650 were known to share a deletion at the *spl1* locus. In the study, F2045 and G9799 are also shown to share a deletion encompassing the *spl1* locus. The overlapping deletions between the four mutant lines delimited the *spl1* locus to only 20 gene models. In the Bruce et al. study, a cytochrome P450 family member was identified as a candidate for *Spl1*.

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term problems caused by crop monoculture and overuse of broad-spectrum pesticides. "Pests thrive where biodiversity is at peril," says Heong. "Instead of genetic engineering, why don't we engineer the ecology by increasing biodiversity?"

Zhu Youyong, president of the Yunnan Agricultural University in Kunming, and his colleagues have found

that growing a mixture of rice varieties across thousands of farms in China could greatly limit the development of rice blast — a fungal rice disease — and boost the yield. They have also tested similar practices using different crops and found beneficial effects.

Debates have also flared because of rice's central role in the Chinese diet. One concern has been that antibiotic-resistance marker genes used

in the derivation of the transgenic plants could invariably be taken up by naturally occurring gut bacteria and lead to resistant, pathogenic strains. Both of Zhu Zhen's Bt/CpTI rice lines and other Bt strains developed elsewhere are free of such markers.

The GM plants must also be shown to be non-allergenic. For food eaten three times a day by a billion people, short-term animal studies aren't



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enough to measure equivalence. "If there were a health risk, we would be heading for a major disaster," warns Liu Bing, an expert on science and society in Tsinghua University in Beijing.

Another concern is the potential environmental consequences of transgenes escaping from GM rice to its unmodified crop counterparts through cross-pollination. The consensus seems to be that perfect prevention of such events is impossible.

Lu Baorong, a biodiversity researcher at Fudan University in Shanghai, is concerned about gene flow from GM rice to its wild or weedy relatives. Wild-rice plants are undomesticated strains, whereas weedy rice, which is characterized by its seed scattering and dormancy, is thought to originate from rice crops as a result of mutations. Lu's team and another group have shown that the rate of gene flow from GM strains to wild and weedy rice is 3–18% and 0.01–0.5%, respectively. This could threaten the biodiversity of wild rice, which provides a valuable gene pool for rice breeders but is already at the brink of extinction in China. Worryingly, many of the stakeholders, including farmers, bioethicists and environmental groups, aren't being involved in the biosafety evaluation process as spear-

headed by the agricultural ministry. The country hails GM rice as a magic bullet for food-production problems and few dissenting opinions are heard. "The whole process is rather opaque," says David Just, an economist at Cornell University in Ithaca, New York. "China is trying very hard to keep the lid on." Experts who express their concerns are often sidelined. Xue, for example, has been repeatedly excluded from biosafety committees that are assessing GM crops.

Full news story at <http://www.nature.com/news/2008/081015/full/455850a.html>



## Calendar of Events

### JUNE 2009

Sun	Mon	Tue	Wed	Thu	Fri	Sat
	1	2	3	4	5	6
7	8	9	10	11	12	13
14	15	16	NE LA 17 field day	LSU rice 18 Field day	19	20
21	22	23	24	25	26	27
28	29	TX A&M 30 Field day				

### JULY 2009

Sun	Mon	Tue	Wed	Thu	Fri	Sat
			LSU rice 1 Field day	2	RiceCAP 3 Ofc closed	4
5	6	LA rice 7 Field day	8	TX A&M 9 Field day	10	11
12	13	14	15	LA, MO, 16 MS Field day	17	18
19	20	21	22	23	24	25
26	27	28	29	30	31	

#### SCHEDULE OF EVENTS

May—August 2009; Summer 2009 rice field days; various locations.

November 15—19, 2009; Rice Genetics VI Symposium in conjunction with the 7th International Rice Functional Genomics Symposium; Manila, Philippines

August 11—15, 2010; 5th International Rice Blast Conference; Little Rock, AR; <http://www.ars.usda.gov/Main/docs.htm?docid=18212>

#### EVENT DETAILS

For all event details, see the appropriate link at [http://www.ricecap.uark.edu/calendar\\_upcoming.htm](http://www.ricecap.uark.edu/calendar_upcoming.htm)



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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.ricecap.uark.edu](http://www.ricecap.uark.edu)



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