The Arizona Genomics Insitute
welcomes you to the

2nd International Symposium of

Rice Functional Genomics

November 15-17, 2004

The Univeristy of Arizona
Mariott Tucson Univerity Park

Tucson, Arizona, USA

Cover Photo courtesy of The University of Arizona

Welcome to the 2nd International Symposium on Rice Functional Genomics!

Rice (*Oryza sativa* L.) is the most important human food crop in the world. The agronomic importance of rice, its shared evolutionary history with major cereal crops, and small genome size have led to the generation of a high-quality finished genome sequence by the International Rice Genome Sequencing Project (IRGSP, 2004, unpublished) as well as three draft sequences by Monsanto, Syngenta and the Beijing Genomics Institute. The highly accurate and public IRGSP sequence now serves as a unifying research platform for a complete functional characterization of the rice genome. Such analysis will investigate the rice transcriptome, proteome and metabolome, with the goal of understanding the biological function of all rice genes (40-50,000) and applying that information to improve rice production and quality. This comprehensive analysis will utilize a variety of techniques and resources from expression and genome tiling arrays to collections of tagged mutant populations developed in elite cultivars grown around the world.

Comparative genomics between the cereal genomes and within the genus *Oryza* will also play a critical role in our understanding of the rice genome. By comparing genome organization, genes and intergenic regions between cereal species, one can identify regions of the genome that are highly conserved or rapidly evolving. Such regions are expected to yield key insights into genome evolution, speciation and domestication. The study of conserved noncoding sequences (CNSs) between cereal genomes will also increase our ability to understand and isolate cis-regulatory elements required for precise developmental and temporal gene expression.

With the inaugural meeting held in Shanghai, China in November 2003, this symposium is the 2nd in what we hope will be an annual international event at different locations throughout the world. In order to achieve our common goal of functionally characterizing all rice genes, we must utilize meetings like this one to develop strong collaborative and multidisciplinary research networks across the world. It is also critical that all data and resources be shared and publicly available without restrictions. Ultimately, our work must be translated into practical solutions that rice breeders can use to improve and stabilize the world food supply for generations to come.

As Chair of the organizing committee, I have had the privilege of working with a dedicated group of scientists and staff to put this meeting together. I would like to thank the international and local organizing committees for helping to identify key speakers for the symposium and organizing the program. I would also like to thank all of the public and private agencies that have provided funding for this meeting. Finally I want to give special thanks to Teri Rambo Mueller, Kiran Rao, Carole Turner, Wendy Gomez, Mario Marquez, Jennifer Currie and members of the AGI/AGCoL/Plant Science staff for taking care of all of the little (and big) details to make this meeting a success.

Enjoy the meeting and your stay in Tucson!

Rod A. Wing

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International Organizing Committee

Chair: Rod A. Wing University of Arizona

Members:

Gyheung An - Pohang University
Michel Delseny - University of Perpignan
Xing-Wang Deng - Yale University
Elizabeth Dennis - CSIRO Plant Industry
Gurdev Khush - University of California, Davis
Hei Leung - International Rice Research Institute
Zhikang Li - International Rice Research Institute
Andy Pereira - Plant Research International
Arjula Reddy - University of Hyderabad
Pam Ronald - University of California, Davis
Takuji Sasaki - National Institute of Agrobiological Sciences
Samuel Sun - The Chinese University of Hong Kong
Akhilesh Tyagi - University of Delhi South Campus
Ray Wu - Cornell University
Yongbiao Xue - Chinese Academy of Sciences
Qifa Zhang - Huazhong Agricultural University

Local Organizing Committee

Members:

David Gang - University of Arizona
David Galbraith - University of Arizona
Scott Jackson - Purdue University
Rich Jorgensen - University of Arizona
Marc Orbach - University of Arizona
Karen Schumaker - University of Arizona
Cari Soderlund - University of Arizona
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Agenda
Sunday, November 14, 2004

Registratrion: 13:00-18:00
Mariott Tucson University Park Lobby

Monday, November 15, 2004

Opening Session: 8:00 - 8:10

Rod Wing: Opening remarks
Gene Sander, Dean College of Agriculture & Life Sciences: Welcome address

Session 1 **Plenary Lectures**
Chair: Rod Wing

8:10 - 8:50 Gurdev S. Khush - Rice Breeding: Past, Present, and Future

8:50 - 9:30 Neil Rutger - The future of rice research in the United States from a breeding perspective

9:30 - 10:00 Coffee / Tea Break

Session 2 **Status of the IRGSP Genome Sequence: Physical Mapping, Sequencing and Annotation**
Chair: Takuji Sasaki

10:00-12:10 Chromosomes 3, 5, 11 and 12

Yeisoo Yu -	Key Elements of Chromosome 3 - USA(12 minutes)
H.H. Chen -	Key Elements of Chromosome 5 - Taiwan(12 minutes)
Akhilesh Tyagi -	Key Elements of Chromosome 11 - India/USA(12 minutes)
Francis Quetier -	Key Elements of Chromosome 12 - France (12 minutes)

Whole Genome

Jianzhong Wu -	The IRGSP Physical Map (12 minutes)
Takahashi Matsumoto -	The RGP Rice Genome Annotation Data Base (40 min)
Robin Buell -	The TIGR Rice Genome Annotation Project(30 minutes)

Group Photo
Lunch 12:10 - 13:30

Session 3 **Status of the Rice Genome - Whole Genome Analysis**
Chair: W. Richard McCombie

13:30 - 14:00 Gane Ka-Shu Wong (Beijing Genomics Institute)- The Ge nomes of Oryza sativa: A history of duplications

14:00 - 14:30 Jiming Jiang (University of Wisconsin) - Structure of centro meric chromatin associated with rice chromosome 8

Coffee / Tea Break 14:30-14:50

Session 4 **Evolutionary and Comparative Genomics**
Chair: Scott Jackson

14:50 - 15:20 Barbara Hass-Jacobus (Purdue University) - Comparative analysis of Oryza sativa (AA) chromosome 1 and *O. brachyantha*

15:20 - 15:45 Michael Purugganan (NCSU) - Evolutionary genomics of rice: SNPs and linkage disequilibrium

15:45 - 16:10 Mark Sorrells (Cornell University) - Comparative DNA se quence analysis of mapped wheat ESTs reveals complexity of genome relationship between rice and wheat

16:10 - 16:35 Ken Wolfe (University of Dublin, Ireland) - Paleopolyploidy and different fates of duplicated genes in different species

16:35 - 17:00 Nori Kurata (National Inst. of Genetics, Japan)- Comparative genomics of expressed sequences between BB, CC and AA genomes in rice

Reception, Dinner, Poster Session - Madera and Pima Rooms - 18:00-20:00

Workshop 1: **Gene tagging - resources, prospects, problems and challenges**

20:00 - 23:00 - Sabino Room
Moderators: Narayana Upadhyaya and Liz Dennis, CSIRO Plant Industry, Canberra, Australia

Contributors: Hirohiko Hirochika (NIAS, Japan); Venkatesan Sundaresan (UC Davis, USA); Emmanuel Guiderdoni/ Pietro Piffanelli (CIRAD France); Andy Pereira (PRI, Netherlands), Qifa Zhang/ Wu Changyin (HAU Wuhan China), Ray Wu (Cornell University, USA), Moo Young Eun/CD Han (RDA.Korea); Narayana Upadhyaya/Liz Dennis (CSIRO Plant Industry, Australia); Ramachandran Srinivasasn (Temasek Life Sciences Laboratory, Singapore); Sumay Yu, Yue-ie C. Hsing (Academia Sinica, Taiwan, in absentia); Chengcai Chu, Qian Qian, Yongbiao Xue (Institute of Genetics & Devel opmental Biology, CAS; Institute of Rice Research, CAAS, China, in absentia).

Workshop Format: The workshop will start with a brief general introduction (overview) on gene tagging, various methods and on the current status by a moderator. This will be followed by individual presentations by the contributors (6-8 minutes each) grouped by the tagging systems. Each presentation will cover the following:- (a) tagging system (eg. T-DNA, Ac/Ds, En/I, Tos17 or any other), (b) construct design and salient features (gene trap/enhancer trap facility, knockout, activation, trans-activation, FST recovery system, excision markers, reinsertion markers or any other special features) and IP status, (c) cultivar used, (d) tagging/trapping efficiency, (e) current collection status, target and timeline, and (f) collaborations. After each group of presentations there will a general (floor) discussion.

Tuesday, November 16, 2004

Session 5 **Functional Genomics I - Mutagenesis and Mutant Collections in Rice**
Chair: Hirohiko Hirochika

8:00 - 8:25 Ray Wu (Cornell University) - A Systematic Approach to Construct an Indexed, Saturation, Insertional-Mutant Rice Library

8:25 - 8:50 Elizabeth Dennis (CSIRO) - Molecular basis of rice cold tolerance

8:50 - 9:15 Darshan Brar (IRRI) - Wild species of Oryza: A valuable genetic resource for rice breeding and functional genomics

9:15 - 9:40 Xing Wang Deng (Yale University)– A comparative tiling-path of transcription activity of *japonica* and *indica* rice chromo some 10 for better understanding of chromosomal architec ture and gene annotation

Coffee / Tea Break 9:40 - 10:00

Session 6 **Functional Genomics II - Genetics, Breeding & Signaling**
Chair: Shiping Wang

10:00 - 10:25 David SanKoff (University of Ottawa, Canada) - Rates of genome rearrangement

10:25 - 10:50 Venkatesan Sundaresan (UC Davis) - Strategies for efficient tagging with heterologous transposons Ac-Ds and En/Spm from maize

10:50 - 11:15 Jiayang Li (Institute of Genetics and Developmental Biology, CAS) - Plant Cell Wall Biosynthesis in Rice

11:15 - 11:40 Susan McCouch (Cornell University) - Fine mapping of a grain weight QTL on rice chromosome 3

11:40 - 12:00 Akhilesh Tyagi (University of Delhi) - Functional Validation of Novel Genes Involved in Abiotic Stress Response and Devel opment in Rice

Lunch **12:00 - 13:30**

Session 7 **Functional Genomics III - Genetics, Breeding & Signaling**
Chair: Emmanuel Guiderdoni

13:30 - 13:55 Qifa Zhang (Wuhan, China) - Genomics approaches to improv ing nitrogen use efficiency of rice

13:55 - 14:20 W. Richard McCombie (CSHL) - Systematic determination of the rice gene set

14:20 - 14:45 Narayana M. Upadhyaya (CSIRO) - Dissociation (DS) inser tional mutagenesis using the transiently expressed transposase: Improved constructs and their suitability for targeted saturation mutagenesis

14:45 - 15:10 Zhi-kang Li (IRRI, CAAS) - Response to selection (drought) and the genetic networks underlying drought tolerance in rice

15:10 - 15:30 Apichart Vanavichit (Kasetsart University, Thailand) - Inte grated functional gnomics in breeding rice for high quality and enriched nutrition

Coffee Break 15:30 - 15:50

Session 8 **Plant-Microbe Interactions**
Chair: Marc Orbach

15:50 - 16:20 Marc Orbach (U. Arizona) - A Genomics Approach to Pathoge nicity: Saturation Insertional Mutagenesis in *Magnaporthe grisea*

16:20 - 16:45 Pamela Ronald (UC Davis): Signaling in the rice XA21-mediated defense response

16:45 - 17:10 Guo-Liang Wang (Ohio State University): Characterization of rice defense mutants using whole genome expression profiling

17:10 - 17:35 Jan Leach (Colorado State University): Approaches To Durable Resistance: Transfer Of Resistance Genes Between Cereal Species

17:35 - 18:00 Yulin Jia (USDA-ARS Stugartt, AR) - Molecular mechanisms of durable rice blast resistance

Dinner - On your own **18:00 - 20:00**

Workshop 2: **Leveraging National and International Collaboration through IRFGC: current agenda and new initiatives**
Chair: Hei Leung

20:00 - 22:00 - Pima and Sabino Rooms

20:00 - 20:15 Hei Leung: Overview of International Rice Functional Genomics Consortium: Setting goals, Steering Committee, USAID Linkage Program to strengthen IRFGC

20:15 – 21:00 Country/Institute updates (~ 5 minutes each)

US –	Jan Leach
Korea –	Moo Young Eun
Japan –	Hirohiko Hirochika
China –	Qifa Chang
France –	Emmanuel Guiderdoni
Australia	Liz Dennis
CIAT	Mathias Lorieux
Singapore	Ramachandran Srinivasan
Others	

21:15 – 21:30 New Initiatives
Narayana Upadhyaya -Summary of Gene Tagging Workshop the way forward

Andy Pereira – Phenotyping network
Perlegen Sciences/IRRI – SNP Project

21:30 – 21:45 Gene Expression Data
Connecting gene chip platforms
Sharing and comparing data

21:45 – 22:00 Hei Leung – Wrap up and open discussion

Wednesday, November 17, 2004

Session 9 **Functional Genomics IV - Proteomics and Metabolomics**
Chair: David Gang

8:00 - 8:25 Nijat Imin (Australian National University) - Proteomic Analysis of Male Gametophyte Development and Its Response to Low Temperature Stress in Rice

8:25 - 8:50 Torsten Kleffmann (Swiss Federal Institute of Technology) - Organelle Proteomics of the Rice Etioplast to Chloroplast Development Reveals Insights into RegulatoryMechanisms of Plastid Biogenesis

8:50 - 9:15 Mark Lange (Washington State University) - Tools and ap proaches for surveying the metabolic capabilities of rice

9:15-9:40 Paul Haynes (University of Arizona) - Functional proteomics of orphan proteins in rice

9:40-10:00 Samuel S.M. Sun (Chinese University of Hong Kong) - Genomic study on the grain quality of hybrid rice

Coffee / Tea Break 10:00 - 10:20

Session 10 The Future of Rice Genomics Research
Chair: Rod A. Wing

10:20 - 10:50 Richard Nelson (Noble Foundation) - Virus-Induced Gene Silencing in Rice for Gene Function Determination

10:50 - 11:20 Blake Meyers (University of Delaware) - Deep transcriptional profiling of rice using MPSS

11:20 - 11:50 Vicki Chandler - (University of Arizona) – Epigenetic control of gene expression

11:50 - 12:30 Joseph Ecker (Salk Institute) - Systematic genome-wide screens in Arabidopsis

12:30 - 12:40 Rod Wing (University of Arizona): Closing Remarks and Announcement of Location and Chair of Next Meeting

Lunch 12:40-14:00

Tour Sonora Desert Museum and Banquet

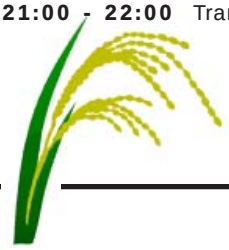
14:00 - 14:45 Transport to Desert Museum

14:45 - 17:00 Tour Desert Museum

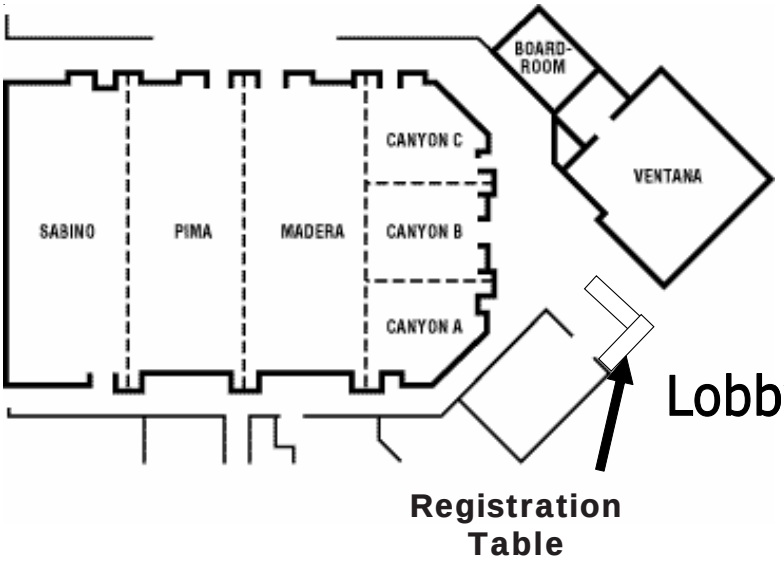
17:00 - 18:00 Reception - Light Refreshments

18:00 - 20:00 Banquet

21:00 - 22:00 Transport to Hotel



Mariott Tucson University Park
Floor Plan



General Information

Registration

Meeting registration will be held in the lobby of the Mariott Tucson University Park. The registration table will be open Sunday at 3 -6 pm and throughout the meeting.

Name Badges

Please wear your name badge at all times. Your badge identifies you as an attendee or exhibitor. You will not be admitted into events without your badge.

Opening Reception

The opening reception will be Monday evening, from 6 to 8 pm in the Ballroom of the Marriott University Park Hotel, with hors d'oeuvres and dinner

Location

All events, except for the banquet on Wednesday, will take place in the Ballroom of the Marriott University Park Hotel. The banquet on Wednesday evening will be held at the Arizona-Sonora Desert Museum.

Banquet at Arizona-Sonora Desert Museum

The banquet will be held Wednesday at the Arizona-Sonora Desert Museum. Vans to the Desert Museum will depart from the front of the Marriott Hotel at 2:00 pm on Wednesday.

Located adjacent to Saguaro National Park at the edge of Tucson (approximately 15 miles from campus), the Arizona-Sonora Desert Museum is a world-renowned zoo, natural history museum, and botanical garden, all in one place. Within the Museum grounds, you will see more than 300 animal species and 1,200 kinds of plants. There are almost 2 miles of paths traversing 21 acres of beautiful desert. The Museum has gained a worldwide reputation in the scientific community as an institution committed to researching and protecting the land, plants, and animals of the Sonoran Desert region.

We will arrive at the Desert Museum around 3:00pm. You are invited to explore the museum before the reception begins. There will be a reception near the Ocotillo Restaurant beginning at 5:00 pm, and dinner will be served at 6:00 pm.

Other meals

A continental breakfast will be served in the area adjacent to the Ballroom in the Marriott Hotel Monday, Tuesday and Wednesday morning beginning at 7:00 am. Attendees will be on their own for lunches on Monday, Tuesday and Wednesday , and for dinner on Tuesday. There are numerous restaurants within walking distance of the Marriott Hotel (see restaurant listings below).

Poster Sessions

There is a poster session during the Monday night reception and the posters will be available for viewing Sunday through Tuesday night. Presenters should set up their posters Sunday evening or Monday morning in the Madera Room and stand by their poster during the Monday reception.

Weather

The weather in Tucson in November is often sunny and pleasant during the day, but may be quite cool at night. It is good to bring a sweater or light jacket when going out in the evening.

Discussion

Each talk will be 20-25 minutes, followed by 5 minutes of discussion. To facilitate discussion, microphones will be available in the audience. We encourage participants to stand and introduce themselves before asking a question.

Tucson and Surrounding Areas

Trolley

There is a trolley that connects the University of Arizona to the Fourth Avenue commercial district. This trolley runs Fridays 6 to10 pm, Saturdays noon until midnight, and Sundays noon to 6pm. The trolley stop is located at the intersection of University Ave. and Tyndall Ave., one block from the Marriott. Fare is $1.00 each way.

The Old Pueblo Trolley passes a variety of shops and restaurants. Beginning in the heart of the Fourth Avenue business district, the trolley turns onto University Boulevard and passes homes, boutiques, cafes, and the Arizona Historical Society, terminating near the new University Park Marriott Hotel and the Main Gate of the University of Arizona.

Taxicabs

The easiest way to get to other areas in Tucson is by taxi. Allstate Cab: 798-1111, Yellow Cab: 624-6611.

Restaurants within walking distance of the Marriott

There are numerous restaurants adjacent to the Marriott. Most are located on University Ave. or on Park Ave. In general, these establishments are inexpensive and cater to students. Some favorites include Pei Wei (Chinese), Frog n Firkin (sandwiches, salads, and pizza), Chipotle (Mexican), Joel's (French), the Fat Greek (Greek), Sinbad's (Mediterranean), Gentle Ben's (brewery and burgers), No Anchovies (pizza), La Salsa (Mexican) and Johnny Rockets (American). These are all located on University Avenue within the same two-block stretch. In addition, there are several inexpensive restaurants along Fourth Avenue, a 15- minute walk from the Marriott, including La Indita (Mexican), Maya Quetzal (Guatemalan), Time Market (sandwiches and pizza, on University Ave, near Fourth Ave).

Restaurants a little further away

The list below includes some of our favorite restaurants. Some of the descriptions are provided by the restaurants themselves, and reservations are recommended at this time of the year. None is within walking distance of the Marriott. Dress in Tucson is casual at most restaurants.

Arizona Inn $$$$
Description: Beautiful hotel and restaurant in central Tucson. Classic and contemporary cuisine with international flavors
Location: 220 E Elm St
Phone number: 520-325-1541

Barrio $$$
Description: A downtown bistro, serves contemporary dishes using fresh local ingredients. Their menu includes pastas, steaks, fish, sandwiches, vegetarian items, and scrumptious desserts. Voted best upscale bar in a local readers poll.
Location: 135 South Sixth Avenue. Just south of Broadway
Phone number: 520-629-0191

Beyond Bread $
Description: Good local sandwich shop and bakery. Excellent breads, sandwiches, salads, and pastries.
Location: 3026 N Campbell Ave
Phone number: 520-322-9965

Bistro Zin $$$
Description: This French inspired American bistro and wine bar has won awards for their wine list and deserts. Bistro Zin's chic intimate interior, cozy patio and hip atmosphere have made it one of the better places to wine and dine in Tucson. 100 wines are available by the glass.
Location: 1865 E River Rd
Phone: 520-299-7799

Café Poca Cosa $$
Description: Chef-owner Susana Davila creates exciting recipes inspired by different regions of her native Mexico, at this innovative Mexican restaurant. The menu, changes daily, and each table gets a stack of warm corn tortillas and a bowl of beans to share.
Location: 88 E Broadway Blvd
Phone: 622-6400

Cuvee $$$
Description: New local favorite, with mix of European and Southwestern cuisine and a good assortment of wines.
Location: 3352 E Speedway
Phone: 881-7577

El Charro $$
Description: Celebrating 80 years of taste and history, El Charro Café of Tucson, Arizona has been called one of the Top 50 Plates in America by USA Today. Featuring Sonoran inspired Tucson-style Mexican Food.
Location: 311 N Court Ave
Phone: 622-1922

Elle $$
Description: A relaxed local restaurant with a diverse menu, very good food, and a commendable wine list. Offerings range from sandwiches to risotto to daily specials including fresh fish and pastas. Relaxed, casual atmosphere.
Location: 3048 E Broadway
Phone: 327-0500

Fuego Restaurant Bar & Grill $$
Description: Fuego offers flambe appetizers, fresh seafood, game and ostrich, mesquite grilled steak, prickly pear pork tenderloin. Wine Spectator Award of Excellence five years in a row.
Location: 6958 E Tanque Verde Rd
Phone number: 886-1745

The Grill at Hacienda del Sol $$$$
Description: The Grill at Hacienda Del Sol is consistently ranked of the top restaurants in Tucson. Located at the beautiful old Hacienda del Sol resort.
Location: Hacienda del Sol Rd.
Phone number: 529-3500

Janos $$$$
Description: Arguably the best (and most expensive) restaurant in Tucson, featuring interesting and inspired Southwestern dishes.
Location: 3770 East Sunrise
Phone: 615-6100

Mi Nidito $$
Description: Typical Mexican fare, located in South Tucson. This is where Clinton ate when he visited Tucson as President. Order the President's platter to get a sense of Clinton's appetite.
Location: 1813 S. 4th Ave.
Phone: 622-5081

Oven's Bistro & Wine Bar $$
Description: Great local restaurant with some Southwestern dishes, less pricey than others.
Location: 4280 N Campbell Ave #37
Phone: 577-9001

Pastiche $$
Description: American cuisine with world influences features appetizers, salads, sandwiches, pastas, large entrees, and desserts.
Location: 3025 N. Campbell Avenue
Phone number: 325-3333

Rosa's Mexican Food $$
Description: Voted Best causal dining, Sonoran Mexican cuisine, carne seca, enchilads, burros and chimichangas (Rod Wing's favorite)
Location: 1750 E Fort Lowell Road
Phone number: 325-0362

Soleil $$$
Description: Great casual French Mediterranean restaurant situated in foothills of the Catalina mountains. Be certain to ask for a table with a view overlooking the city.
Location: 3001 E Skyline Drive
Phone: 299-3345

Sushi Saga $$
Description: A Mexican sushi restaurant, with great sushi, fish tacos and other interesting Mexican-Japanese combinations. Very casual, a local favorite.
Location: 2955 E. Speedway
Phone: 320-0535

Sulivan's $$$
Description: Upscale steakhouse. Large portions, good steak.
Location: 1785 E. River Rd.
Phone: 299-4275

Vivace $$$
Description: Chef Daniel Scordato, highly regarded in Tucson for his fantastic Italian cuisine, has created a stellar menu full of favorites like crispy crab cannelloni, linguini with grilled salmon, and veal osso bucco.
Location: 4310 N. Campbell
Phone: 795-7221

Yoshimatsu $
Description: Inexpensive Japanese restaurant (but not mainly sushi) with lots of vegetarian options. Serves donburi (rice bowls), miso soup, curries, okonomiyaki (a sort of omlet or pancake), noodles, and a wide assortment of bento (mixed dinner boxes). Uses only organically grown vegetables.
Location: 2660 N Campbell Ave
Phone: 320-1574

Points of interest within walking distance of the Marriott

Arizona State Museum. The oldest and largest anthropology museum in the Southwest. Experience the indigenous cultures of Arizona, the Greater Southwest, and northern Mexico. Dynamic exhibitions, engaging programs, a renowned research library, and an educational museum store engage visitors of all ages. ASM's experts and collections are among the most significant resources in the world for the study of southwestern peoples. Hours Monday-Saturday 10-5, Sundays noon-5. 1013 E University Blvd, on campus, three blocks from the Marriott.

Center for Creative Photography. CCP is an archive, museum, and research center dedicated to photography as an art form and cultural record. CCP's vast collection includes more archives and individual works by 20th-century North American photographers than any other museum in the nation. The gallery is open 9-5 Monday-Friday and noon-5 on weekends. CCP is located on campus, 1030 North Olive Road, a few blocks from the Marriott. Phone: 621-7968.

Flandrau Science Center. Science museum and planetarium. Located on the University of Arizona campus at 1601 E University (about seven blocks from the Marriott). Hours: Monday-Saturday 9-5 pm, Sunday 1-5 pm. Also open 7-9 pm Thursday, Friday, and Saturday.

Fourth Avenue Commercial District. A lively area with many shops and bars. To get there from the Marriott, go West on University a few blocks, then turn left on Fourth Ave. The trolley also goes there (see above).

University of Arizona Campus. Adjacent to the Marriott. Includes many desert plants and offers a nice quiet place to walk.

Points of interest in and around Tucson

Arizona-Sonora Desert Museum. See description under "General Information".

Sabino Canyon Recreation Area. Nestled in the foothills of Arizona's southern Catalina Mountains 12 miles from downtown Tucson, the oasis of Catalina Canyon is one of the most scenic spectacles in Arizona. A paved road runs 3.8 miles into the canyon, crossing 9 stone bridges over Sabino Creek. It begins at an altitude of 2,800 feet and rises to 3,300 feet at its end, a popular drop-off in summer because of the swimming holes at Hutch's Pool and The Crack. Winding through the canyon, visitors who follow the road have views of the creek, the riparian vegetation, magnificent Saguaros on the canyon walls, and towering rock formations. Picnic areas are scattered along the road, as are trailheads leading to other sections of the National Forest or paralleling the road.

Saguaro National Park. Bordering Tucson on both the East and West, this National Park includes great hiking trails and easy access to the Sonoran Desert. For more information call: Visitor Information - Rincon Mountain District (520) 733-5153; Visitor Information - Tucson Mountain District (520) 733-5158; Headquarters (520) 733-5100.

San Xavier Mission. The San Xavier del Bac Mission is one of the oldest and most well preserved missions in the southwest. Located just off Interstate Hwy 19 at Exit 92, on the San Xavier Indian Reservation, 6 miles south of Interstate10 in Tucson. Catholic services are held every Sunday and are open to the public. Address: 1950 W San Xavier Rd. Phone: 520-294-2624.

Tohono Chul Park. Tohono Chul Park is a center where nature, art, and culture connect. Includes nature trails, botanical gardens, exhibits in a renovated historic home, a greenhouse where you can buy many native plants, and a restaurant. The Park's mission is to enrich people's lives by providing them the opportunity to find peace and inspiration in a place of beauty, to experience the wonders of the Sonoran Desert, and to gain knowledge of the natural and cultural heritage of this region. 7366 N Paseo del Norte, (520) 742-6455. 8-5 daily.

Tucson Botanical Garden. Tucked within the heart of the city, Tucson Botanical Gardens is a five-acre collection of 15 specialty gardens including a historical garden, an herb garden, a butterfly garden, a cactus and succulent garden, and much more. The collection consists of over 4,200 individual plants. They offer many design ideas appropriate to the scale of most residential gardens. Come visit the urban oasis and discover the variety of plants that thrive in southern Arizona. 2150 N Alvernon Way, Phone: (520) 326-9686.

Pima Air and Space Museum. The Pima Air & Space Museum (PASM) features over 200 aircraft on display and has five large hangars totaling 100,000 square feet of exhibit space. An original WWII barracks contains an extensive model collection, arranged chronologically, which shows virtually all U. S. military aircraft from pre-World War I to the present. 6000 East Valencia Road, Phone: (520) 574-0462

Reid Park Zoo. Travel from the mountains of South America, through the Asian rain forest, and to the African savanna in just a few hours. The Reid Park Zoo encourages visitors to explore naturalistic habitats exhibiting over 500 exotic animals from around the world. Corner of 22nd St. and Lakeshore Dr; between Country Club and Alvernon, Phone: (520)791-4022

Restaurant Guide

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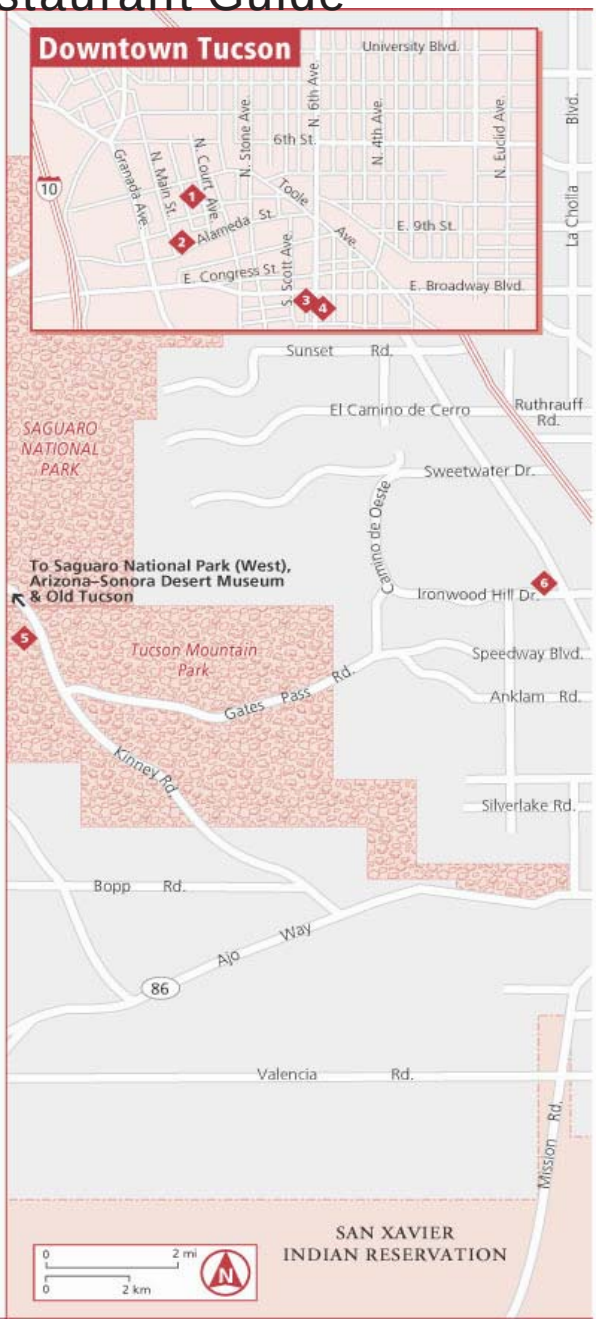
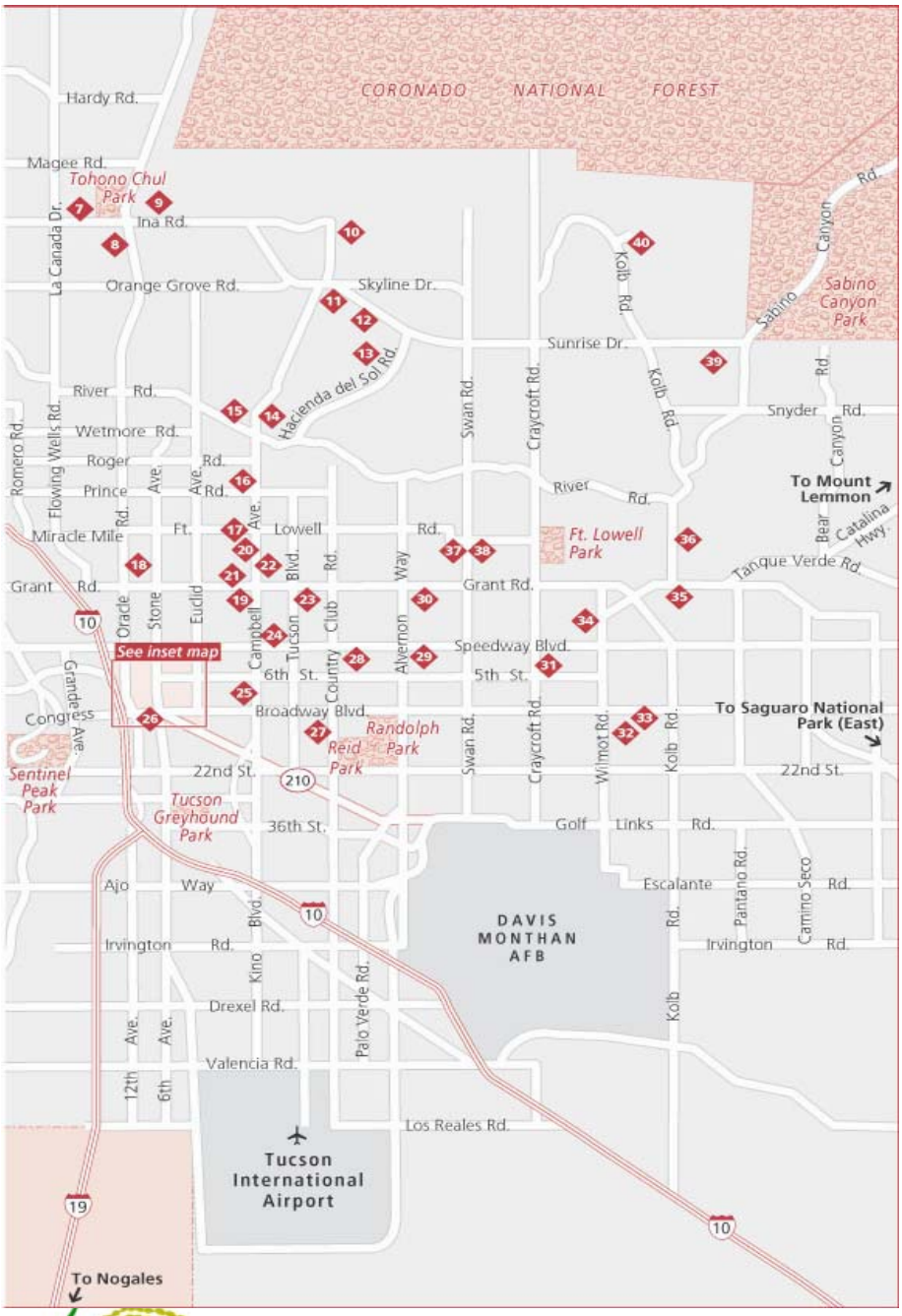
Tohono Chul Tea Room **7**
Ventana Room **40**
Vivace Restaurant **16**
Wildflower **8**
Yoshimatsu Healthy Japanese Food & Café **19**





Attraction Guide

- Arizona Historical Society Museum Downtown **9**
- Arizona Historical Society Tucson Museum **12**
- Arizona State Museum **13**
- Arizona-Sonora Desert Museum **1**
- Center for Creative Photography **11**
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- El Tiradito **21**
- Flandrau Science Center & Planetarium **14**
- Gadsden-Pacific Toy Train Operating Museum **3**
- Magic Carpet Golf **7**
- Mission San Xavier del Bac **18**
- Old Tucson Studios **2**
- Pima Air & Space Museum **17**
- Reid Park Zoo **16**
- Sabino Canyon **6**
- Sosa-Carillo-Frémont House Museum **20**
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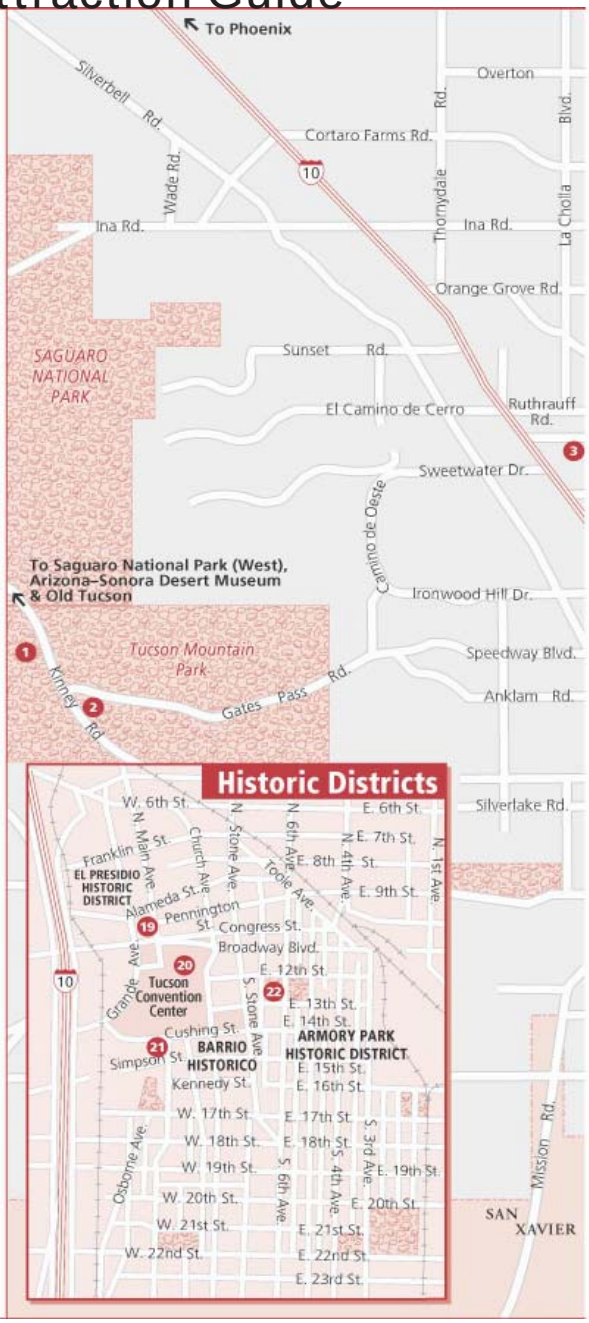
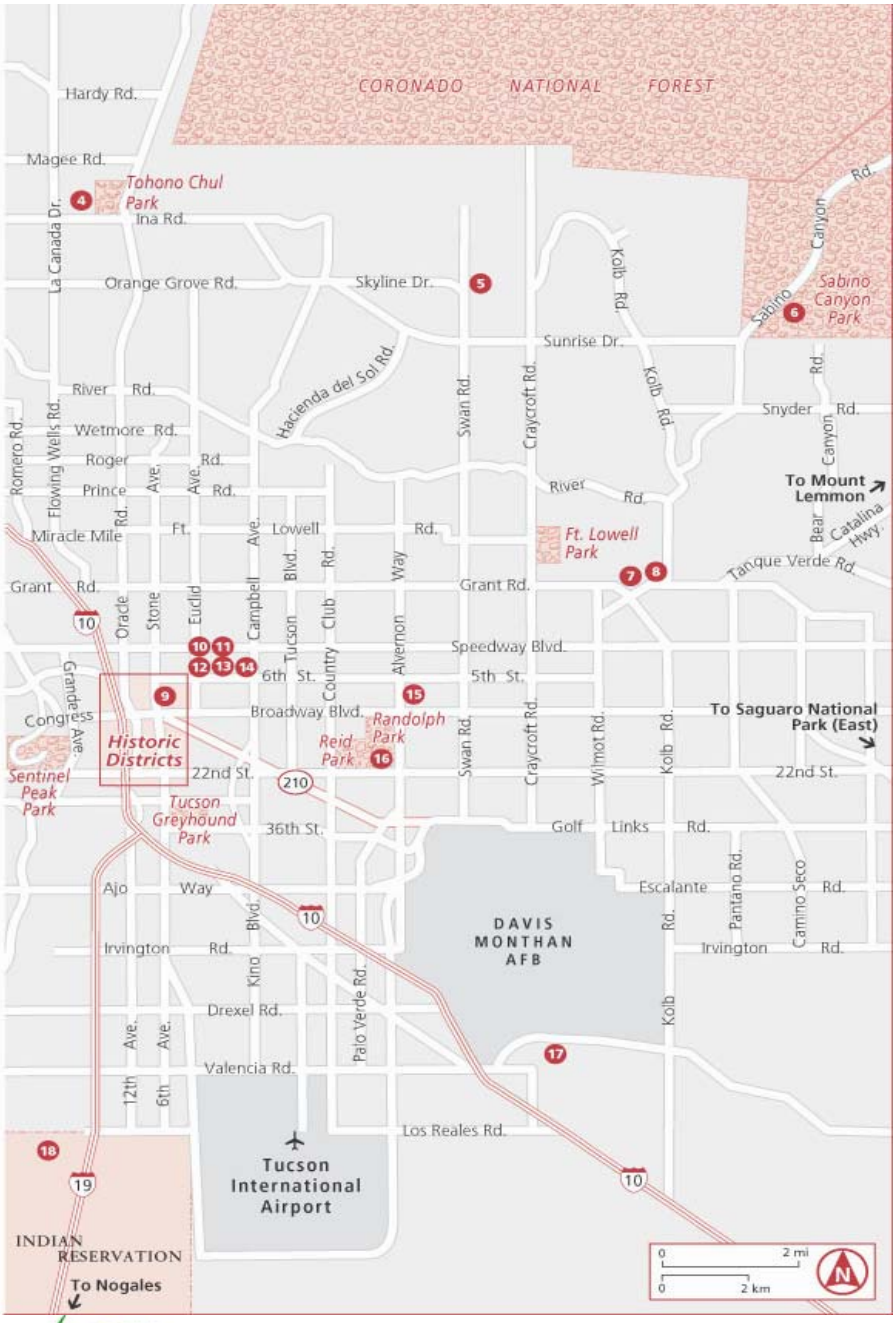
- Tucson Botanical Gardens **15**
- Tucson Children's Museum **22**
- Tucson Museum of Art & Historic Block **19**
- University of Arizona Museum of Art **10**





Session 1
Plenary Lectures
Chair: Rod Wing

Gurdev S. Khush - Rice Breeding: Past, Present, and Future

Neil Rutger - The future of rice research in the United States from a breeding perspective

SPEAKER

RICE BREEDING: PAST, PRESENT, AND FUTURE

Gurdev S. Khush

University of California, Davis, CA 95616

Rice was first domesticated about ten thousand years ago polyphyletically in Southern China and Himalayan foothills in India. Constant human selection for improved traits led to development of about 150,000 land races in different parts of the world. In the beginning of last century, rice breeding stations were established in China, India and Japan and in other major rice growing countries somewhat later. To begin with rice breeders made pure line selections from heterogeneous land races. Inter-Varietal hybridization started during 1930s. However, major efforts on rice improvement started after the establishment of the International Rice Research Institute (IRRI) in the Philippines in 1960s. Emphasis on yield improvement led to release of IR8 in 1966. IR8 had improved plant type with short stature, sturdy stems and dark green and erect leaves. Improved plant type resulted in doubling of rice yield potential. Other important traits such as good grain quality, shorter growth duration, multiple resistance to diseases, insects, and tolerance to some abiotic stresses were incorporated into IR8 plant type. Most of the national rice improvement programs (NARS) followed suit and started developing improved plant type germplasm. Improved plant type varieties developed by IRRI and NARS are now planted to about 80% of the world rice area. This resulted in doubling of rice production in a 35 year period and led to food security.

Population of rice consumers is increasing at the rate of 1.8% annually. Moreover 4oo million rice consumers still go to bed hungry everyday. If poverty alleviation programs succeed, their purchasing power will increase and so will the demand for rice. It is estimated that we will have to produce 38% more rice from existing land resources by 2030 to feed 5 billion rice consumers. To meet this challenge we need rice varieties with higher yield potential and greater yield stability. Conventional plant breeding approaches a well as new techniques of molecular biology are being employed to develop rice varieties. These include molecular marker aided selection and genetic engineering. Ongoing work on functional genomics will help identify many useful genes for developing rice varieties in the future.

SPEAKER

THE FUTURE OF RICE RESEARCH IN THE UNITED STATES FROM A PLANT BREEDING PERSPECTIVE

J. Neil Rutger

USDA-ARS Dale Bumpers National Rice Research Center, Stuttgart, AR, 72003

Rice breeding invariably has involved and will continue to involve one or more of the "Big Four" goals common to all crop breeding programs: yield, quality, pest resistance, and stress tolerance. Standard methods — pedigree, backcrossing, induced mutation — will continue to be the backbone, although they may be revolutionized, possibly in ways that today are inconceivable. Certainly the standard methods will be supplemented by molecular technologies and expansion of multidisciplinary research teams. For example, the recent establishment of the RICECAP is expected to aid in development of biotechnology-based tools to enhance two characters that have been difficult to improve through conventional breeding, milling quality and sheath blight disease resistance.

Higher yields are considered essential for virtually all crops, including rice. At 7.4 mt/ha, US rice yields on its 1.2 million hectares are well above the world average of 3.8 mt/ha on 153 million hectares. In fact US national rice yields are exceeded only by those of Australia at 8.6 mt, in an environmental production area of 150,000 hectares which is favored by the same type of Mediterranean climate enjoyed by California, which has similar or higher yields on 200,000 hectares. It is essential that pursuit of ever-higher yields continue, in order to keep the US rice industry competitive in the global marketplace.

Rice breeders in the US focus on grain quality, especially since the US rice industry has a well-earned reputation for producing the best quality rice in the world. However, the grain quality of US rice has not gone unnoticed by rice breeders in Asia, many of whom are pursuing the very same quality factors considered important for US rice: translucent long grains with intermediate amylose levels and high whole-grain milling yields which constitute some 80% of southern long grain production, and translucent medium grains with low amylose levels and high whole grain milling yields which constitute virtually all of California production. And, in fact in production of aromatic rices Asian rices are considered superior to current US varieties, resulting in imports of 14% of the rice being eaten in this country today, up from zero imports two decades ago. Therefore most US breeding programs now include development of aromatic rices.

The US, and indeed the entire western hemisphere, is fortunate to have relatively few of the traditional disease and insect pests common to Asian rice production. A restrictive quarantine import procedure, which often frustrates US rice scientists, is an integral key to keeping out introduced diseases and pests. Within the US, continued progress is being realized on developing blast resistant varieties, and efforts on developing sheath blight resistance are intensifying. Among insect pests, several programs continue to seek resistance to the rice water weevil, an insect indigenous to the US. Resistance to another insect, rice stink bug would be desirable, but sources of major resistance have not been found.

Stress tolerance research is being pursued for improved cold tolerance, especially in California, and in Arkansas. Promising levels of improved tolerance to straighthead, which is often referred to as a physiological disease and is a problem in some southern rice areas, have been found in newer indicas. Drought tolerance, important in many rainfed areas of the world is not a major objective in the US, since high production costs dictate that rice must be grown under optimal conditions, which means full flood. Salinity tolerance is pursued from time to time, but is not a major breeding objective in the relatively land-rich US rice production areas.

The germplasm base of US rices is relatively narrow, and as recently as 1990 all southern US varieties traced back to 22 accessions, while California varieties traced back to 23 accessions. In recent years some broadening of germplasm has occurred, almost all in the tropical japonica long grains, or in the temperate japonica medium grains. Only one indica variety, Jasmine 85 is grown in the US, and that on a limited area. In the last two decades some rather spectacular yield increases, as well as better disease resistance, have been observed in indicas from China, although grain quality is not acceptable for US markets. Therefore a base-broadening effort, concentrating on indicas, has been launched at the Dale Bumpers National Rice Research Center.

SPEAKER

Session 2
Status of the IRGSP Genome Sequence: Physical Mapping, Sequencing and Annotation
Chair: Takuji Sasaki

Yeisoo Yu - Key Elements of Chromosome 3

H.H. Chen - Key Elements of Chromosome 5

Akhilesh Tyagi - Key Elements of Chromosome 11

Francis Quetier - Key Elements of Chromosome 12

Jianzhong Wu - The IRGSP Physical Map

Takahashi Matsumoto - The RGP Rice Genome Annotation Data Base

Robin Buell - The TIGR Rice Genome Annotation Project

SPEAKER

KEY ELEMENTS OF RICE CHROMOSOME3

The Rice Chromosome 3 Sequencing Consortium

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Cold Spring Harbor Lita Annenberg Hazen Genome Sequencing Center, Cold Spring Harbor, NY 11797 USA
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Washington University Genome Sequencing Center, St. Louis, MO 63108 USA

Rice is the most important food crop in the world and is considered a model system for plant biology because of its evolutionary relationship with other important cereals (e.g., maize, barley and wheat) and its compact genome. In 1998, the International Rice Genome Sequencing Project (IRGSP) was initiated to determine the complete genomic sequence of rice (*Oryza sativa* ssp. *japonica* cv Nipponbare) and is now in its final stages of finishing the genome by December 2004.

Chromosome 3 has been estimated at 39Mb (~166cM) in physical length and it is considered one of the most gene-rich chromosomes of rice. ACWW and TIGR recently finished sequencing 331 minimum tiling BAC/PAC clones across rice chromosome 3 and constructed a pseudomolecule which represents 36.1Mb of non-redundant sequence with five physical gaps. Annotation of the pseudomolecule identified over 6,200 gene models and about 21% of the chromosome contains transposable elements and other types of repetitive sequences. Sequence analysis between rice chromosome 3 and other cereals (maize and wheat) also revealed highly preserved syntenic blocks across the chromosome. In addition, we reconstructed a BAC/STC-based physical map of the rice chromosome 3 with the progenitor of cultivated japonica rice, *O. nivara*, which allowed us to estimate the level of divergence between these two closely related species. Annotation results and detailed sequence analysis of chromosome 3 will be presented.

SPEAKER

RICE CHROMOSOME 5: PHYSICAL MAPPING, SEQUENCING AND ANNOTATION

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Academia Sinica Plant Genome Center (ASPGC)

Institute of Botany, Academia Sinica, Taipei 115, Taiwan

A fine physical map of the rice (*Oryza sativa japonica* Nipponbare) chromosome 5 with bacterial artificial chromosome (BAC) and PI-derived artificial chromosome (PAC) clones was constructed through an integration of 279 sequenced BAC/PAC clones and 232 STS/EST markers with fingerprinted contig data of the Nipponbare genome. This map consists of 7 contigs covering 99% of the estimated chromosome size (30.02 Mb). The sizes of the six physical gaps were estimated as 26 kb, 28 kb, 30 kb, 32 kb, 49 kb, and 23 kb for gaps 1, 2, 3, 4, 5 and 6 respectively. We has submitted 42.2 Mb sequences with 29.73 Mb of non-overlapping sequences to public databases. Extension of the flanking sequence within 5 physical gap regions was obtained by screening of a 40-kb-insert fosmid library. Among these gaps, two gaps (gaps 1 and 5) were closed through this effort. BAC clones corresponding to telomere and centromere regions were confirmed by the use of BAC-fluorescence *in situ* hybridization (FISH) on pachytene chromosome. The genetically centromeric region at 54.6 cM was covered by a minimum tiling path spanning for 2.1 Mb with no physical gaps. The centromeric core was composed of 3 overlapping BAC/PACs that constitute a staggered signal for ~150 kb when used as FISH probes. In addition, uneven condensation at the pericentromeric region on chromosome 5 was detected. All the finished BACs were annotated according to the integrated results of the following: BLASTN2.0, BLASTX2.0, GENSCAN Fgenesh GlimmerR, TWINSCAN and GeneSplicer. The sequence was searched against the Swiss-Prot+TrEMBL protein database, the NCBI Plant EST database, the TIGR Rice Gene Index and the rice full-length cDNA database (KOME, http://cdna01.dna.affrc. Go.jp/cDNA/). The annotation effort is still underway.

SPEAKER

ANALYSIS OF THE SEQUENCE OF LONG ARM OF CHROMOSOME 11 FROM RICE

A. K. Tyagi[1], J. P. Khurana[1], Paramjit Khurana[1], S. Raghuvanshi[1], A. Mohanty[1], A. Bharti[1], A. Gaur[1], V. Gupta[1], D. Kumar[1], V. Ravi[1], S. Vij[1], A. Kapur[1], Parul Khurana[1]

K. Gaikwad[2], A. Singh[2], V. Dalal[2], S. Srivastava[2], A. Dixit[2], A. K. Pal[2], I. A. Ghazi[2], M. Yadav[2], A. Pandit[2], A. Bhargava[2], Sureshbabu K. [2], K. Batra[2], S. D. Mendiratta[2], V. Singhal[2], P. K. Singh[2], H. Singh[2], T. R. Sharma[2], T. Mohapatra[2], N. K. Singh[2]

T. Sasaki[3], J. Messing[4], R, Wing[5], W. R. McCombie[6]

[1]IIRGS, DPMB, University of Delhi South Campus, New Delhi-21, India
[2]IIRGS, NRCPB, Indian Agricultural Research Institute, New Delhi-12, India
[3]Rice Genome Program, NIAS, Tsukuba, Japan
[4]PGIR, Waksman Institute, Rutgers, NJ, USA
[5]AGI, Tucson, AZ, USA
[6]Cold Spring Harbor Laboratory, NY, USA

The pseudomolecule for chromosome 11 of rice is 28.3 Mb, as estimated from the assembled sequence. The sequencing of the region from 57.3 to 116.2 cM, on the long arm, was assigned to India. This task was accomplished by sequencing 118 BAC/PAC/Fosmid clones to generate 16.5 Mb high quality sequence at 10X level and less than 1 error in 10,000 bases; this corresponds to ca. 12.68 Mb of non-overlapping sequence. A single gap of ~43 kb at 79 cM was estimated by fiber-FISH with adjoining BAC clones and extended sequence of adjoining Fosmid clones. The overall GC content turns out to be 43.3% although exons are rich in GC (51.6%). The region is expected to have 2453 gene models, 607 being TE related genes. The non-TE gene models (1846) show a gene density of one gene/6.9 kb, which is better than the average gene density of chromosome 11 or whole rice genome. A large number (~400) of these genes lack introns and average number of exons per gene is four. About 20% of these gene models showed extended homology to the genome of indica rice. A large number of full-length cDNA (414) and ESTs from rice, maize, sorghum and wheat match gene models. Out of the rice genes showing significant homology to wheat ESTs, 113 could be mapped to bins located on various wheat chromosomes, the largest number being on homeologous group 4 chromosomes. Such a wide distribution pattern can be ascribed to the dynamic state of rice and wheat genomes causing significant loss of colinearity.

SPEAKER

The IRGSP Physical Map

Jianzhong Wu

Rice Genome Research Program (RGP), National Institute of Agrobiological Sciences / Institute of the Society for Techno-innovation of Agriculture, Forestry and Fisheries. 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan.

The International Rice Genome Sequencing Project (IRGSP), established in 1998, is a world-wide consortium of scientists to attain the accurate and complete sequence of the rice genome (*Oryza sativa* ssp. *Japonica* cv. Nipponbare). Having members from 10 countries and regions (Japan, US, China, Taiwan, France, India, Korea, Thailand, Bracil and UK), the IRGSP adopted the clone-by-clone shot-gun sequencing strategy and completed a map-based and high quality draft sequence in December 2002. After the publication of draft sequence, IRGSP continued its efforts to construct the physical map towards the goal of a complete rice sequence. Three centromere regions (Chr4, 5 and 8) were mapped and sequenced. In addition, maps and sequences of 7 telomeres were obtained. The 45S rDNA (Chr9) and 5S rDNA (Chr11) gene clusters were also partially sequenced. As a result, the total number of physical gaps has been reduced two-fold to 62. Near half of these gaps are associated with the centromere or telomere regions. With the FISH technology, physical sizes of all gaps were measured, that revealed only 18 Mb of the rice genome was not covered by the IRGSP physical map. About 80% of these gaps are supposed to be heterochromatic regions. In summary, the current IRGSP map has 370.7 Mb in length that covers 95.3% of the whole genome and virtually all of the euchromatin. The IRGSP now uses the completed sequence from the above physical map for extensive analysis of rice genome such as gene annotation, that will no doubt provide fundamental knowledge for us to understand and manipulate the biology of rice, one of the most important crops in the world.

SPEAKER

THE RGP RICE GENOME ANNOTATION DATABASE

Matsumoto,T., Namiki,N., Ito,Y-I., Mukai, Y., Ito,Y.,Tsugane,M., Shibata,M.,Yamamoto,M., Hosokawa.S., Hamada,M., Negishi,M., Sakata,K., Numa, H., Antonio,B.,Nagamura,Y., and Sasaki,T.

Rice Genome Research Program (RGP), National Institute of Agrobiological Sciences / Institute of the Society for Techno-innovation of Agriculture, Forestry and Fisheries,Japan

The International Rice Genome Sequencing Project (IRGSP) is about to accomplish its mission of elucidating the complete sequence of the rice genome. More than 350 Mb of accurate rice genome sequence has already been submitted to public databases. This high-quality sequence will serve as a gold standard for understanding the functions of individual genes and the gene-network involved in expression of complex agricultural traits. Since it is now widely known that rice has syntenic relationships with other grasses, the rice genome sequence will also be extremely useful for the analysis of other major cereal crops such as maize, barley and wheat. Moreover, the accurate genome sequence will be the key to detect the gene duplication, segmental duplication, and polyploidity among the domesticated cultivars and between the wild and domesticated rice species with the eternal goal of understanding how rice genomes evolved, diverged, and domesticated during these 10-12 million years.

The RGP is in-charge of sequencing chromosomes 1, 2, 6, 7, 8 and 9, corresponding to almost half of the entire genome. Among 1809 PAC/BAC clones which comprised the minimum tiling of these six chromosomes, a total of 1798 clones have been submitted to DDBJ in the PLN category as of October 2004. The total length of the non-redundant sequence is approximately 190 Mbp. For the annotation of the finished clone sequences, we have adopted a speedy annotation using our auto-annotation system, RiceGAAS (http://ricegaas.dna.affrc.go.jp/) and an accurate, evidence-based annotation followed by map-based presentation on INE database (http://rgp.dna.affrc.go.jp/giot/INE.html). The RiceGAAS incorporates new analysis tools, resources and database so that the annotation is updated on a regular basis. The manually curated gene models on INE provide evidence of homology to rice full-length cDNA sequences and ESTs. These gene models are then aligned in the contig-based annotation database RAD (http://rad.dna.affrc.go.jp/) that aims to provide a genome-based, comprehensive annotation to understand the biological importance of individual genes based on chromosomal position. So far, the manual curation of annotation for chromosomes 1, 2, 7, 8 and 9 has been completed, and chromosome 6 will be finished soon as well. The accurate gene models will be useful for global identification of agronomically important genes for efficient application in rice breeding.

SPEAKER

THE TIGR RICE GENOME ANNOTATION PROJECT

C. Robin Buell, Brian Haas, Charles Lu, Rama Maiti, Shu Ouyang, Aihui Wang, Jennifer Wortman, Qiaoping Yuan, and Wei Zhu

The Institute for Genomic Research, Rockville, MD 20850

In January, 2004 we began a four-year project funded by the National Science Foundation to annotate the rice genome. This project will expand on our previous annotation efforts in rice. The foundation of this project is an annotation database for rice (osa1) that houses sequence and annotation information. To date, we have identified gene models in rice using a combination of ab initio gene finders and experimental evidence. We have constructed pseudomolecules of the 12 rice chromosomes and provided these, along with our annotation, as a public resource for rice and cereal biologists. Currently, a second version (Release 2) of our pseudomolecules is available to the public through our project web site (rice.tigr.org). We have been performing assessments and improvements to our annotation pipeline to refine the quality of annotation. We have incorporated full length cDNA and EST evidence into gene models and improved our gene name assignment methods. We generated a series of other annotations for the rice genome including gene ontology assignments, domains, motifs, and alignment with tagged insertion sequences. We have also developed a series of alignments with other plant species including alignments with ESTs and with genetic markers from maize and wheat. We have expanded our web-based tools and will be releasing a Data Extractor and an Integrated Genome Browser in the near future. We are on track to update our annotation to incorporate more finished sequence from the International Rice Genome Sequencing Project and our improved annotation methodologies by January 2005. All of our data are available via the project website: rice.tigr.org.

SPEAKER

Session 3
Status of the Rice Genome - Whole Genome Analysis
Chair: W. Richard McCombie

Gane Ka-Shu Wong (Beijing Genomics Institute) - The Genomes of Oryza sativa: A history of duplications

Jiming Jiang (University of Wisconsin) - Structure of centromeric chromatin associated with rice chromosome 8

SPEAKER

THE GENOMES OF ORYZA SATIVA: A HISTORY OF DUPLICATIONS

Gane Ka-Shu Wong, Jun Yu, Jian Wang, and Huanming Yang

Beijing Institute of Genomics, Chinese Academy of Sciences

We report improved whole-genome shotgun (WGS) sequences for the genomes of indica and japonica rice, both with multi-megabase contiguity, or almost a factor of 1000 improvement over the drafts of 2002. Tested against a non-redundant collection of 19,079 full-length cDNAs, 98.1% of the genes are aligned, without fragmentation, to the mapped super-scaffolds of one or the other genome. We introduce a gene identification procedure for plants that does not rely on similarity to known genes to remove erroneous predictions due to transposable elements. Using the available EST data to adjust for residual errors in the predictions, the estimated gene count is at least 38,000~40,000. Only 2% to 3% of the genes are unique to any one subspecies, comparable to the amount of sequence that might still be missing. Despite this lack of variation in gene content, there is enormous variation in the intergenic regions. At least a quarter of the two sequences could not be aligned, and where they could be aligned, single-nucleotide-polymorphism (SNP) rates varied from as little as 3.0 SNP/kb in the coding regions, to 27.6 SNP/kb in the transposable elements. A more inclusive new approach for analyzing duplication history is introduced. It reveals an ancient whole-genome duplication, a recent segmental duplication between chromosomes 11 and 12, and massive ongoing individual gene duplications. We find 18 distinct pairs of duplicated segments that cover 65.7% of the genome, and 17 of these pairs date back to a common time before the divergence of the grasses. More importantly, ongoing individual gene duplications provide a never-ending source of raw material for gene genesis, and are major contributors to the differences between members of the grass family.

SPEAKER

STRUCTURE OF CENTROMERIC CHROMATIN ASSOCIATED WITH RICE CHROMOSOME 8

Jiming Jiang

University of Wisconsin, Madison, WI 53706

Abstract: The centromeres of complex eukaryotic species are composed of highly repetitive DNA sequences. Satellite DNA and transposons are the most common DNA elements in centromeres. Therefore, it has been a major challenge to map, clone and sequence centromeres in complex eukaryotic species. We have demonstrated that the centromeres of rice chromosomes contain a 155-bp satellite repeat, CentO, and a centromere-specific retrotransposon, CRR. We recently sequenced the centromere of rice chromosome 8, which contains only ~60 kb CentO satellite. A ~740 kb region within the centromere binds rice CenH3, the centromere-specific H3 histone. Histones H3 and CenH3 are interspersed within the 740 kb region. Several active genes were found in the CenH3-binding domain, suggesting that the cytologically distinctive centromeric chromatin is suitable for gene expression. This euchromatic characteristic of the CenH3-binding domain has been verified by its association with H3 methylated at lysine 4. We propose that the centromere of rice chromosme 8 may represent an intermediate stage as centromeres evolve from genic regions to fully mature centromeres that often contain megabase arrays of homogeneous satellite DNA.

SPEAKER

Session 4
Evolutionary and Comparative Genomics
Chair: Scott Jackson

Barbara Hass-Jacobus (Purdue University) - Comparative analysis of Oryza sativa (AA) chromosome 1 and
O. brachyantha

Michael Purugganan (North Carolina State University) - Evolutionary genomics of rice: SNPs and linkage disequilibrium

Mark Sorrells (Cornell University) - Comparative DNA sequence analysis of mapped wheat ESTs reveals complexity of genome relationship between rice and wheat

Ken Wolfe - (University of Dublin, Ireland) - Paleopolyploidy and different fates of duplicated genes in different species

Nori Kurata (National Institute of Genetics)- Comparative genomics of expressed sequences between BB, CC and AA genomes in rice

SPEAKER

COMPARATIVE ANALYSES OF *ORYZA SATIVA* (AA) CHROMOSOME 1 IN *O. BRACHYANTHA* (FF)

Jackson, S.A., Gill, N., SanMiguel, P., Stein, L., Wing, R., Abernathy, B., Westerman, R., Walling, J.G., Hass-Jacobus, B.

Purdue University, West Lafayette, IN 47907

Oryza brachyantha is a ~343 Mb FF genome diploid relative of cultivated rice, O. sativa. Using contigged BACs, BAC end sequences (BES), overgos derived from O. sativa, we recapitulated rice chromosome 1 in O. brachyantha and evaluated repetitive sequence content from the BES relative to rice. Rice chromosome 1 is ~42.9 Mb and accounts for ~1/10 of the rice genome. Of 64,566 O. brachyantha BES, 1,386 mapped to rice chromosome 1 using Blat. Approximately 4% of chromosome 1 was recovered using only paired BES, but 86% was recovered using both paired and single BES. BACs from O. brachyantha were also fingerprinted and contigged and using both overgo hybridization and BES we aligned these contigs to chromosome 1. Analysis of the BES revealed several repetitive sequences that, using FISH, were found to be either dispersed or tandem and mostly pericentromeric in origin. This is the first step toward the recapitulation of an entire rice chromosomes in related Oryza species that will be used to understand mechanisms of chromosome evolution at the genus level.

SPEAKER

EVOLUTIONARY GENOMICS OF RICE: SNPS AND LINKAGE DISEQUILIBRIUM

Purugganan, M.D., Caicedo, A.L., Olsen, K.M., Mather, K.A., Wilson, G., Born, D., Polato, N., Nierlsen, R., Bustamante, C., McCouch, S.

North Carolina State University, Raleigh. NC 27695
Cornell University, Ithaca, NY 14853

Rice is a recognized as a model system for the study of cereal crop genomes. Despite its position as the world's predominant cereal food crop and as a model for grass genomics research, relatively little about the evolutionary forces that shape genomic diversity in rice. The study of the evolutionary genomics of rice, specifically levels and patterns of linkage disequilibrium (LD) and single nucleotide polymorphisms (SNPs) provides the foundation for improved genome scanning and mapping techniques to isolate quantitative trait loci that can be used for crop improvement in rice, as well as in other selfing crop species. Understanding the patterns of SNP correlations and diversity is also necessary for the construction of a whole genome rice haplotype map (HapMap). The extent of linkage disequilibrium in ten targeted 500-kb genomic regions will be determined in O. sativa subspecies indica and japonica, as well as the progenitor wild species O. rufipogon. Patterns of LD will be compared between regions of different recombination rates and between neutral loci and genes subjected to selection under domestication. The levels and patterns of single nucleotide polymorphisms will also be analyzed in a set of randomly chosen gene fragments to determine the distribution of population genetic parameters in rice. Here, we present preliminary data on the levels and patterns of SNP variation in rice, and the extent of linkage disequilibrium in one genomic region.

SPEAKER

COMPARATIVE DNA SEQUENCE ANALYSIS OF MAPPED WHEAT ESTS REVEALS COMPLEXITY OF GENOME RELATIONSHIPS BETWEEN RICE AND WHEAT

Sorrells, Mark E. and La Rota, Mauricio

Cornell University, Ithaca, NY 14853

Comparative sequence analyses can be used to cross reference genes between species maps, enhance the resolution of comparative maps, study patterns of gene evolution, and facilitate interspecies gene cloning. We used BLASTn to compare 5780 Triticeae ESTs that had been previously mapped using wheat deletion lines, to the rice genome sequence from 3280 ordered BAC/PAC clones. A rice genome view of the homologous wheat genome locations based on sequence analysis shows general similarity to the previously published comparative maps based on Southern analysis of RFLP but the much higher resolution exposed numerous breaks in colinearity. The inverse view showing the relationship between the wheat deletion map and the rice genomic sequence further revealed the breakdown of gene content and order at the resolution conferred by the physical chromosome deletions in the wheat genome. Wheat deletion bins, even in the most conserved regions, often contained sequences that matched those from multiple rice chromosomes. This suggests that since the evolutionary divergence of wheat and rice, there has been an abundance of rearrangements, insertions, deletions, and duplications eroding the wheat-rice microsynteny that may complicate the use of rice as a model for cross-species transfer of information, especially for the less conserved regions. The enhanced resolution afforded by this comparative DNA sequence analysis will benefit wheat researchers by facilitating the selection of markers for saturation mapping and the selection candidate genes for association analysis. Grass genomes appear to be evolving more rapidly and in different ways than previously thought.

SPEAKER

PALEOPOLYPLOIDY AND DIFFERENT FATES OF DUPLICATED GENES IN DIFFERENT SPECIES

Wolfe, K.H., Scannell, D.R., Byrne, K.P., Gordon, J., Wong, S.

University of Dublin, Trinity College, Dublin, Ireland

Abstract: Polyploidy has played a significant role in the evolution of most plant species. There is good evidence from comparative genomics that numerous ancient genome duplications (paleopolyploidizations) occurred at various points during plant evolution, including one in the shared ancestor of rice, maize and wheat. When a gene becomes duplicated as the result of a polyploidization, both copies can survive in the genome or one copy can be lost again. What determines the outcome? And if one copy is lost, which one? We are studying this process using yeast genomes as a model system. A whole-genome duplication occurred in the common ancestor of *Saccharomyces cerevisiae* and several other yeast species, about 100 million years ago. Subsequently, most of the duplicated genes were quickly lost again. The "choice" of which loci remain duplicated and which return to single-copy seems to involves both some general principles and some species-specific aspects of biology. Genes involved in signal transduction were preferentially retained in duplicate in all yeasts (and also after paleopolyploidy in Arabidopsis). However, at many other types of loci the sorting-out process proceeded differently in different lineages. For example, many transcription factors that are single-copy in *S. cerevisiae* are retained in two copies in *S. castellii*. Most surprisingly, at more than 100 loci that survived in duplicate up to the time that *S. cerevisiae* and *S. castellii* diverged, these two species subsequently lost different copies of the locus. The result is that each species retains only one copy of the gene, but these are paralogs (not orthologs) and are located at homeologous (not orthologous) positions in the genome. Differential loss of alternative copies of duplicated genes has the potential to cause reproductive barriers between species via a Bateson-Dobzhansky-Muller chromosomal incompatibility mechanism.

SPEAKER

COMPARATIVE GENOMICS OF EXPRESSED SEQUENCES BETWEEN BB, CC AND AA GENOMES IN RICE.

Kurata,N., Sano,Y., Eiguchi,M., Kanamori,H., Yamazaki,Y.

National Institute of Genetics, Japan

It should be attractive for functional genomics studies of rice to compare genome sequences between different genomes or species and also between close relatives. This will make it possible to identify genome or species specific regions on the chromosomes, divergent genes among species and variant genes/alleles among varieties. Another way to identify genes varying in sequence or specific to species is by cDNA analysis and their comparison between species. We are progressing in the analysis of cDNAs for *O. punctata*, BB genome species, and *O. officinalis*, CC genome species, to compare their sequences with those of AA genome. Though the analysis is still at a preliminary stage, it showed several categories of genes with variation in sequences between genomes and they ranged from a high similarity group to no similarity group with the AA genome sequence. A few to many scores of base substitutions with synonymous or non-synonymous amino acid changes were detected in almost all genes. Many insertions/deletions (InDels) were also detected both in the coding and the 5' and 3' untranslated regions. Some examples and status of the difference in gene sequences will be presented and discussed.

In addition to the sequence analysis of cDNAs, a microarray analysis was performed to compare expression profiles between AA and BB, and between AA and CC. The 22K 60mer microarray of O, sativa (AA genome) was examined by applying RNAs from shoot apical meristem (SAM) and very young influorescence. Most genes showed much higher expression in AA compared to BB and CC, indicated missing oligomer sequences from the transcripts of BB and CC, but some genes were found to express higher in BB and CC than AA. Characterization of these genes might partly explain the genome specificity achieved by the difference of gene expression.

SPEAKER

Workshop 1
Gene tagging – Resources, Prospects, Problems and Challenges
Chairs: Narayana M. Upadhyaya & Liz Dennis

Contributors

Hirohiko Hirochika (NIAS, Japan)

V. Sundaresan (UC Davis, USA)

Emmanuel Guiderdoni/ Pietro Piffanelli (CIRAD, France)

Andy Pereira (PRI, Netherlands)

Qifa Zhang/ Wu Changyin (HAU Wuhan, China)

Ray Wu (Cornell University, USA)

Moo Young Eun/CD Han (RDA, Korea)

N.M. Upadhyaya/Liz Dennis (CSIRO Plant Industry, Australia)

Ramachandran Srinivasasn (Temasek Life Sciences Laboratory, Singapore)

In absentia

Summary Yu, Yue-ie C. Hsing (Academia Sinica,Taiwan), Chengcai Chu, Qian Qian, Yongbiao Xue (Institute of Genetics & Developmental Biology, CAS; Institute of Rice Research, CAAS, China)

SPEAKER

Session 5
Functional Genomics I - Mutagenesis and Mutant Collections in Rice
Chair: Hirohiko Hirochika

Ray Wu (Cornell University): A Systematic Approach to Construct an Indexed, Saturation, Insertional-Mutant Rice Library

Elizabeth Dennis (CSIRO) - Molecular basis of rice cold tolerance

Darshan Brar (International Rice Research Institute) - Wild species of Oryza: A valuable genetic resource for rice breeding and functional genomics

Xing Wang Deng (Yale University)– A comparative tiling-path of transcription activity of *japonica* and *indica* rice chromosome 10 for better understanding of chromosomal architecture and gene annotation

SPEAKER

A SYSTEMATIC APPROACH TO CONSTRUCT AN INDEXED, SATURATION, INSERTIONAL-MUTANT RICE LIBRARY

Cheng-Kun He, Moul Dey, Faping Duan, Fengling Li, Zhihong Lin, Jin Su, Ajay Garg and Ray Wu,

Cornell University, Ithaca, NY 14850

Our goal is to employ a systematic approach for the construction of an indexed, saturation, insertional-mutant rice library, that includes both activation trap and gene trap features, for functional genomics. The approach is unique in that it can be used to generate an Ac/Ds-based, transposon-tagged rice mutant population that will help to speed up gene discovery. One major advantage of our novel approach is that a specific chromosomal location can be chosen and produces a truly saturated insertional-mutant library with an insertion every 1.6 kb, on average. The other important advantage is that in this mutant library, the approximate chromosomal location of each mutant is determined by using a rapid PCR-based high-throughput procedure. Thus, for the reverse genetic approach of locating a specific insertional-mutant corresponding to a specific gene of interest, it will only be necessary to screen several plant lines, rather than an entire mutant library.

Our systematic approach involves the following steps, and pilot experiments showed that each step works.

(1) To construct a Ds-containing plasmid that can serve for both activation trap and gene trap; it contains a selectable marker (hyg) for transformation of rice, an excision marker (bar) to detect transposition, a truncated rice cytochrome c gene (C108), for rapid PCR-based determination of the copy of integrated plasmid in the rice genome.

(2) To produce 800 potentially useful anchor lines, each harboring a single copy of the Ds plasmid, evenly distributed throughout the rice genome, from which to choose (after pilot experiments) 200 productive anchor lines, with an average distance of 2,200 kb between anchor lines.

(3) To construct an Ac-containing plasmid that contains an Ac-transposase gene from maize driven by a ubiquitin promoter.

(4) To carry out pilot experiments to choose productive Ds-anchor lines among potentially useful anchors and active Ac plants by crossing six potentially useful Ds-anchor lines each with five different Ac-plant lines. Next, at least 2,000 F2 seeds from each cross combination will be used to obtain preliminary data on transposition frequency. The result will tell us whether a given Ds-anchor line is considered a productive anchor line and which Ac plants can promote high levels of transposition.

(5) To cross each productive Ds-anchor with two pre-tested, active Ac plant lines to generate at least 8,000 F3 transposants. We will then estimate the transposition distance and location of each transposant within a 2,500 kb region by a rapid, two-dimensional, long-PCR-based high-throughput procedure.

(6) To choose from each anchor line approximately 1,600 insertional-mutant sublines that, on average, are 1.6 kb apart from neighboring sublines to saturate a 2,500-kb region that surrounds a specific anchor position. Note that at this stage of the procedure, we convert a randomly tagged library to an indexed, linked, mutant library by using a high-throughput procedure.

(7) To eventually complete the analysis, with participation of many collaborators, of all 200 productive anchor lines to produce a total of 320,000 indexed, insertional-mutant lines.

SPEAKER

MOLECULAR BASIS OF RICE COLD TOLERANCE

E.S. Dennis [1,2], S. Oliver [1,2,3] , J. Van Dongen [4] , P. Geigenberger [4] , H.S. Saini 5 , S. Fernandes [1,2] , C. Blanchard [1,3] , X. Zhao [6] , E.S. Dennis [1,2] , and R. Dolferus [1,2] .

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The young microspore stage of rice pollen development is highly sensitive to cold temperature damage which causes abortion of pollen development and pollen sterility. Australian rice varieties are particularly sensitive to cold-induced pollen sterility. Drought-induced pollen sterility in wheat and rice is associated with disruption in anther sugar metabolism. We show that sugar metabolism is also disrupted in rice anthers by cold stress. Cold temperature at the young microspore stage causes an accumulation of sucrose and reduction in cell wall invertase activity in anthers, and a failure of starch deposition in mature pollen. We have identified four invertase genes and two monosaccharide transporter genes that are expressed in rice anthers. Of these, the cell wall invertase gene OSINV4 appears to be anther-specific, and is down-regulated by cold in anthers. The monosaccharide transporter gene OSMST8 is also down-regulated by cold in anthers, suggesting that a sugar transport pathway involving OSINV4 and OSMST8 could be blocked by cold. The expression of another monosaccharide transporter gene, OSMST7, was induced by cold, which could suggest that cold stress triggers the re-direction of sugars away from the developing pollen. We found that expression of the genes encoding anther cell wall invertase (OSINV4) and monosaccharide transporter (OSMST8) were not repressed to the same extent by cold in two Chinese cold-tolerant rice varieties. We also investigated the possible role of abscisic acid (ABA) as a cause of the cold-induced changes in sugar metabolism. ABA levels increased in anthers in response to cold treatment at the young microspore stage. ABA application to developing panicles mimicked the effect of cold treatment on grain yield and on the expression of the sugar metabolic genes OSINV4, OSMST7, and OSMST8. Our results indicate that an anther-specific synthesis of ABA is associated with pollen sterility, and that ABA could act as a signal for causing sterility, possibly in combination with the changes in sugar metabolism.
We constructed microarrays containing 18,000 anonymous clones from rice anther cDNA libraries. We identified genes in the cold sensitive Australian variety Doongara that are differentially expressed in response to cold at the young microspore stage of anther development. We also compared the cold response at different stages of anther development. The response to cold and water stress demonstrated a significant overlap suggesting that cold and water stress induce pollen sterility by the same mechanism. We used microarray screening to compare the cold response of the two Chinese cold-tolerant varieties to the sensitive line Doongara and found substantial differences.

SPEAKER

WILD SPECIES OF ORYZA: A VALUABLE GENETIC RESOURCE FOR RICE BREEDING AND FUNCTIONAL GENOMICS

D.S. Brar 1, G. S. Khush [1], D. J. Mackill [1], H. Leung [1] and R. A. Wing [2]

[1] International Rice Research Institute, Los Banos, Philippines
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A number of biotic and abiotic stresses reduce rice productivity. There is thus need to broaden the gene pool of rice. Wild species (2n=24-48) of Oryza representing 10 genomes provide a reservoir of genes for tolerance to various stresses. Low crossability, increased sterility, restricted homoeologous pairing and linkage drag limit introgression of genes from wild species. Advances in tissue culture, molecular markers and genomics have enabled gene transfer and precise monitoring and characterization of alien introgression from distant genomes of Oryza. Interspecific hybrids and alien introgression lines have been produced across crossability barriers and monosomic alien addition lines developed. Alien genes for resistance to grassy stunt virus, tungro virus, brown planthopper, bacterial blight, blast, tolerance to acid sulfate conditions and WA cytoplasm (CMS) have been introgressed. Varieties with genes from wild species for resistance to stresses have been released for commercial cultivation. Some of the introgressed alien genes (Bph10, bph11, bph12, Pi9t, Xa21) including QTLs have been tagged. One of the genes (Xa21) for bacterial blight resistance has been pyramided along with other genes through MAS in many rice cultivars. Yield enhancing loci/QTLs from wild species have been identified. Molecular analysis has shown limited introgression of short segments from distant genomes. Parental genomes, alien chromosomes, introgressed segments and homoeologous pairing can be detected precisely through GISH. Autosyndetic pairing detected by GISH is indicative of duplications in the sativa genome. Identification of gene(s) controlling homoeologous pairing would enhance alien introgression from distant genomes. Availability of mapping populations and near isogenic alien introgression lines carrying defined segments from wild species (BBCC, CC, EE, FF) and segmental chromosomal substitution lines (AA genome) are providing important genetic resource for functional genomics. BAC libraries developed under OMAP for each representative genome of Oryza offer new opportunities to isolate agronomically important genes and QTLs for use in rice improvement. BAC libraries of wild species could be screened with one or a few specific gene families involved in biotic and abiotic stress tolerance and the hybridizing BAC clones could be used in transformation of cultivated rice. This would be important to transfer genes from wild species which lack pairing and recombination with the cultivated rice genome.

SPEAKER

A COMPARATIVE TILING-PATH MICROARRAY ANALYSIS OF TRANSCRIPTION ACTIVITY OF JAPONICA AND INDICA RICE CHROMOSOME 10 FOR BETTER UNDERSTANDING OF CHROMOSOMAL ARCHITECTURE AND GENE ANNOTATION

Xing Wang Deng[1], Lei Li[1], Xiangfeng Wang[2, 3, 4], Mian Xia[5], Viktor Stolc [1, 6], Ning Su [1], Zhiyu Peng [2], Songgang Li [3], Jun Wang [4], Xiping Wang [5], and Xing Wang Deng [1] (LL & XW contributed equally to this work)

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SPEAKER

Genome sequencing of the two major subspecies of domestic rice (Oryza sativa), japonica and indica, is essentially completed. The abundant unusual compositional and structural features make accurate annotation of the rice genomes an immediate challenge. We report here a comparative tiling-path microarray analysis of the transcription units of the rice chromosome 10 of both rice genomes. This analysis provides an overall expression support for 2472 (81.9%) of prior 3019 total non-redundant gene models, including 91.9% or 77.6% expression support rates for approximately either half of those gene models with or without prior cDNA or EST support. This chromosome-wide transcription analysis facilitated identification of additional 549 expression supported gene models, an increase of 18.2% in the total coding capacity. A chromosomal-wide cDNA cloning analysis of the new gene models as well as prior gene models without cDNA or EST supports further validates the tiling microarray discovery of transcription units and provides additional insights into rice gene fine structure. The chromosome-wide transcriptional analysis also suggests a functional correlation between chromosome 10 transcription and the chromosomal architecture.

Session 6
Functional Genomics II - Genetics, Breeding & Signaling
Chair: Shiping Wang

David SanKoff (University of Ottawa) - Rates of genome rearrangement

Venkatesan Sundaresan (University of California, Davis): Strategies for efficient tagging with heterologous transposons Ac-Ds and En/Spm from maize

Jiayang Li (Institute of Genetics and Developmental Biology, CAS) - Plant Cell Wall Biosynthesis in Rice

Susan McCouch (Cornell University) - Fine mapping of a grain weight QTL on rice chromosome 3

Akhilesh Tyagi (University of Delhi, India): Functional Validation of Novel Genes Involved in Abiotic Stress Response and Development in Rice

SPEAKER

RATES OF GENOME REARRANGEMENT

David Sankoff

University of Ottawa, Ottawa, Canada

In a comparative map, the number of translocations in the evolutionary history of a chromosome can be estimated solely on the basis of the conserved syntenies it contains. This estimate, subtracted from the number of conserved segments, then allows the estimation of the number of inversions that have affected the chromosome. Summing these estimates over all chromosomes provides an accurate estimator (as assessed by simulation) of the total number of rearrangements of each type occurring in the evolutionary divergence of two genomes.

SPEAKER

STRATEGIES FOR EFFICIENT MUTAGENESIS WITH HETEROLOGOUS TRANSPOSONS AC-DS AND EN / SPM FROM MAIZE

Sundaresan, V., Chellian, S., Persson, A., Buzkan, A., Hogan, P., Qu, S., Radhomony, R., McClinton, R., Chlan, X., Yu, Y., Kim, H., Wing, R.

University of California-Davis, University of Arizona, Grand Valley State University, University of Louisiana-Lafayette

Insertional mutagenesis with transposons is a useful alternative to T-DNA, especially when transformation through tissue culture is inefficient as is the case for many rice species. We are using heterologous transposons from maize, which are well characterized and therefore easily modified to permit efficient identification of transposed elements. The strategies we have developed for mutagenesis of rice require a limited number of primary transformants, with insertions being generated through propagation by selfing. Stable new insertions can be selected through the use of fluorescent marker genes. A single primary transformant can yield 40-80 independent insertion lines after two generations of propagation. Both the Ac-Ds and En/Spm transposon systems were successfully utilized to generate insertion lines in Niponbare through these strategies. Analysis of flanking sequences suggests the En/Spm system is comparable in efficiency to Ac-Ds elements but generates a larger fraction of unlinked transposon insertions, and can provide complementary coverage of the genome.

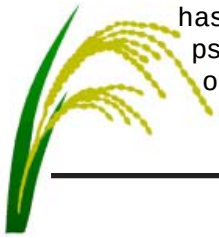
SPEAKER

PLANT CELL WALL BIOSYNTHESIS IN RICE

Jiayang Li, Yihua Zhou, Yunhai Li, Shenben Li, and Zhang Mu

Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China

The plant cell wall, a strong fibrillar network that provides mechanical support to cells, tissues, and the entire plant body, is a highly organized composite that contains many different polysaccharides, aromatic substances, and proteins. Polysaccharides comprise the bulk of wall structural components, being ~90% by dry weight of primary walls and ~60% by dry weight of lignified secondary walls. Plant cell wall biosynthesis, in particular the synthesis of wall polysaccharides and wall assembly, occurs through a complex and intricate series of steps that often begin in an intracellular compartment and end in the wall itself. Regulation of the steps is central to cell development, since the polysaccharide composition of the wall changes during cell division, elongation and differentiation. Till now, how this coordinate regulation of wall biosynthesis is achieved is unknown and remains to be elucidated. To understand the mechanisms that regulate the mechanical strength of the plant body and the biosynthesis of plant cell walls, mutants defective in stem strength have been isolated and characterized. At the molecular level, a number of genes and gene families have been identified, which makes major breakthroughs in our understanding of polysaccharide biosynthesis. In rice, at least six brittle culm mutants (bc1 to bc6) have been reported and some of them were mapped to different chromosomes using classic or molecular approaches. Here, we report the isolation and molecular characterization of rice mutants displaying the brittle culm phenotypes. BC1, which encodes a COBRA-like protein, is expressed mainly in mechanical tissues of rice. Mutations in BC1 cause not only a reduction in cell wall thickness and cellulose content, but also an increase in lignin level, suggesting that BC1 plays an important role in the biosynthesis of the cell walls of mechanical tissues. Domains predicted to play important roles are being studied by transgenic researches. Immunogold and biochemical approaches have revealed that the GPI anchor attachment is necessary for the subcellular localization of BC1 on cell walls. A genome-wide search of DNA and protein databases has identified nine BC1-like genes and one pseudogene and approaches of antisense, RNi and ostrategies have been applied to investigate the functions of all members of this family.



SPEAKER

FINE MAPPING OF A GRAIN WEIGHT QTL ON RICE CHROMOSOME 3

Susan McCouch, Jiming Li, Michael Thomson

Cornell University, Ithaca, NY 14853

As the basis for fine mapping of a grain weight QTL, gw3.1, a set of near isogenic lines (NILs) was developed from an *O. sativa*, cv. Jefferson x *O. rufipogon* (IRGC105491) population based on five generations of backcrossing and seven generations of selfing. The locus was associated with transgressive variation for grain size and grain weight in this population and features prominently in many other inter- and intra-specific crosses of rice. Variance in size and weight among grains on a panicle and between grains on primary and secondary panicles required the use of extreme sampling and NIL group-mean comparisons to evaluate phenotype. Despite the peri-centromeric location of the QTL and the use of an inter-specific cross for mapping, we observed no suppression of recombination in the region and have been able to narrow down the location of the gene underlying this QTL to a 93.8 kb interval. The fact that a QTL for kernel size has also been identified in a homoeologous region of maize chromosome 1 suggests that this locus, in which the dominant O. rufipogon allele confers small seed size, may be associated with domestication in cereals.

SPEAKER

FUNCTIONAL VALIDATION OF NOVEL GENES INVOLVED IN ABIOTIC STRESS RESPONSE AND DEVELOPMENT IN RICE

Akhilesh K. Tyagi, J.P. Khurana, S. Kapoor, S. Raghuvanshi, J.K. Thakur, A. Mukhopadhyay, V. Gupta, S. Anand, H. Kathuria, S. Vij, S. Ray, R. Arora, P. Agarwal and S. Kumar

Department of Plant Molecular Biology, University of Delhi South Campus, New Delhi-110021, India (e-mail: akhilesh@genomeindia.org)

India has maximum area in the world under rice cultivation, ~28% of its arable land, and produces about 135 million tons of rough rice. Looking at growing population and rice yield in India, it is imperative to look for novel means for improving rice yield and protecting losses due to stresses. By differential screening, a gene (OSISAP1) encoding for a stress associated zinc finger protein has been isolated. The gene is induced in response to several abiotic stresses like cold, salt, dehydration, submergence and heavy metals. The overexpression of the gene in tobacco confers tolerance to cold, salt and dehydration at the seedling stage. Genome-wide distribution of its homologs in rice, expression profile as well as their protein-protein interactions have been investigated. Similarly, several anther-specific genes have been isolated and their promoters characterized in transgenics. One of the genes codes for a CDPK, a gene family represented by at least 35 genes in rice. Detailed in silico analysis of this gene family will be presented. These genes have been used for expression profiling by real-time PCR to identify stress-inducible genes as well as genes expressing during flower and seed development. A polycomb group gene, OsiEZ1, has also been characterized from rice, which may be involved in embryo/endosperm development, and could possibly act by chromatin modulation. SAGE analysis has also been performed from young panicles at male meiotic stage to identify new genes of relevance. By comparing ~20,000 tags from flower-specific stage and leaves, several stage-specific genes have been identified. These should form the basis for functional validation.

SPEAKER

Session 7
Functional Genomics III - Genetics, Breeding & Signaling
Chair: Emmanuel Guiderdoni

Qifa Zhang (National Key Laboratory of Crop Genetic Improvement, Wuhan, China) - Genomics approaches to improving nitrogen use efficiency of rice

W. Richard McCombie (Cold Spring Harbor Laboratory) - Systematic determination of the rice gene set

Narayana M. Upadhyaya (CSIRO) - Dissociation (DS) insertional mutagenesis using the transiently expressed transposase: Improved constructs and their suitability for targeted saturation mutagenesis

Zhi-kang Li (International Rice Reasearch Institute, CAAS) - Response to selection (drought) and the genetic networks underlying drought tolerance in rice

Apichart Vanavichit (Kasetsart University, Thailand) - Integrated functional gnomics in breeding rice for high quality and enriched nutrition

SPEAKER

GENOMICS APPROACHES TO IMPROVING NITROGEN USE EFFICIENCY OF RICE

Qifa Zhang[1], Xingming Lian[1], Zemin Zhou[1], Zhuqing Zhao[2], Caiguo Xu[1], Shiping Wang[1], Jianwei Zhang[1], Dejun Yuan[1] and Xianghua Li[1]

[1]National Key Laboratory of Crop Genetic Improvement and [2]College of Resources and Environments, Huazhong Agricultural University, Wuhan 430070, China

Nitrogen is a crucial plant macronutrient and is needed in the greatest amount of all mineral elements required by plants. In the last half a century, global use of N fertilizer has increased more than 10-folds in order to increase crop productivity. Plants consume much less than half of the fertilizers applied, while majority of N fertilizers is lost to the environment, which causes increasingly severe pollutions. Moreover, fertilizer application has now become the major cost in crop production, which greatly affects the income of the farmers. Thus, developing crops that are less dependent on the heavy application of N fertilizers is essential for the sustainability of agriculture. In recent years, we have adopted a combination of genomic approaches to discover genes that are involved in the nitrogen metabolism, with the ultimate goal to improve nitrogen use efficiency (NUE) of the rice crop. The approaches include: (1) screening of germplasm collection for high NUE, (2) identifying and mapping QTLs for high NUE, (3) screening of mutant libraries for identifying genes involved in nitrogen metabolism, (4) microarray analysis of genes differentially induced by low-N stress, (5) modifying the expression of genes known to be involved in nitrogen assimilation, and (6) functional test of candidate genes for potential to improve NUE by transgenic analysis. Substantial progresses have been made with all the approaches, and the current results will be presented in the conference.

SPEAKER

SYSTEMATIC EXPERIMENTAL DETERMINATION OF THE STRUCTURE AND FUNCTION OF GENES IN THE RICE GENOME

McCombie, W. R., Balija, V., Palmer, L.

Cold Spring Harbor Laboratory, Cold Spring Harbor,, NY 11797

Rice is the most important food crop in the world, being cultivated for more than 9,000 years, and is the staple for over 50% of the population. The International Rice Genome Sequencing Project (IRGSP) is on the verge of completing a highly accurate, annotated sequence of the rice genome. At that point the challenge becomes determining the structure and function of the elements within the genome. Our group at Cold Spring Harbor Laboratory have initiated a gene verification/discovery project. In the first phase, 292 Riken full length cDNAs were tested by RACE to check the accuracy of the transcriptional start site annoations of transcripts. 112 of the 292 genes targeted were successfully amplified from total RNA extracted from rice leaves. Of the 112 transcript sequences, the first base of 104 the putative transcript could be mapped to the rice genome. 82 of the 104 targets had a RIKEN rice cDNA that differed from a RACE product by over 10 bases. In only 48 of the 104 targets, there was a RACE product that mapped within 10 bases of the RIKEN cDNA. Further analyses of these and other genes is underway. However, it would seem likely based on experience with similar analyses in mouse, that we may be detecting a high level of alternate splicing in the rice genome. In addition to determining the structures of the gene we have recently begun a project to examine the function of genes in rice. To accomplish this we will be carrying out protein interaction analyses of selected gene products in order to associate those proteins with previously characterized proteins within rice. This will allow us to assign cellular functions to these gene products. The same data will form the beginnings of a rice protein interaction map.

SPEAKER

DISSOCIATION (*DS*) INSERTIONAL MUTAGENESIS USING THE TRANSIENTLY EXPRESSED TRANSPOSASE: IMPROVED CONSTRUCTS AND THEIR SUITABILITY FOR TARGETED SATURATION MUTAGENESIS

Upadhyaya, N.M., Zhu, Q-H., Hoque, M.S., Ramm, K., Shivakkumar, S., Smith, K., Pan, S-T., Li, S., Peng, K., Dennis, E.S.

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Following the completion of the genome sequences of Arabidopsis and rice, the international focus now is to identify specific functions encoded by the predicted ~50,000 plant genes. The obvious method of determining gene function is to isolate mutants of each gene and study the effects of those mutations on the plant. Rice insertion lines are being generated in several laboratories world-wide, using retrotransposons (*Tos17*), T-DNA, *Ac*/*Ds* and *En*/*I*. In particular, the two-component *Ac*/*Ds* or *En*/*I* systems provide the added advantage of being able to remobilize the insertion mutagen to produce new insertion lines. We, and others, have already shown that *Ac*/*Ds*-based gene and enhancer trap systems work in rice yielding ~5% unique stable insertion lines in a given screening population derived either from the crossing of *Ac* and *Ds* lines, or from *Ac*/*Ds* double transformants. Although variable, on average ~60% of the insertions are linked to the original location of *Ds* within the T-DNA (the *Ds* launch pad), with the majority of them landing within 4 cM on either side of the *Ds* launch pad.

We have developed *Ds*-containing T-DNA constructs with the following features: (1) a bi-directional gene trap facility with two different trap reporters, (2) an herbicide (bar) resistance gene as an initial selection marker, and as a Ds reinsertion marker, (3) hygromycin resistance gene (*hph*) as a Ds excision marker, (4) a flanking sequence tag (FST) recovery system based on an E. coli origin of replication and an antibiotic resistance (*bla*) gene, and (5) a killer gene (*barnase*) system to enrich for single-copy clean T-DNA (by eliminating T-DNA with vector backbone or with T-DNA direct repeats). We are producing large number of such clean T-DNA/*Ds* launch pads distributed over all the rice chromosomes.

By super infecting callus tissue from the above mentioned single-copy T-DNA/*Ds* lines with *Agrobacterium* harbouring *Ac* constructs, also containing a visual marker (*gfp*), we have been able to regenerate stable *Ds* insertion lines at a frequency of ~5% in addition to expected *Ac*/*Ds* double transformants, by applying selection pressure (hygromycin) for *Ds* excision. The progeny of the double transformants also yield expected stable insertion lines with appropriate selection and visual marker screening.

The availability of a large number of such clean T-DNA/*Ds* launch pads will facilitate chromosomal region-directed insertion mutagenesis in a high throughput manner to saturate the corresponding region with *Ds* insertions. Saturation insertional mutagenesis of the whole genome is a huge task, requiring serious collaborations among researchers. This system does provide an opportunity for chromosomal region-wise distribution of gene tagging tasks among collaborating laboratories.

SPEAKER

RESPONSE TO SELECTION (DROUGHT) AND THE GENETIC NETWORKS UNDERLYING DROUGHT TOLERANCE IN RICE

Z. K. Li[1,2], R. Lafitte[1], Y. M. Gao[1,2], B. Y. Fu[1,2], J. L. Xu[1,2], C. H. M. Vijayakumar[1], T. Q. Zheng[1,2], J. Ali[1], M. F. Zhao[1], Y. Z. Jiang[1], S. B. Yu[1], J. Domingo[1], R. Maghirang[1], G. S. Khush[1], and D. MAckill[1]

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A large-scale backcross breeding program has been initiated since 1998 to exploit the genetic diversity in the primary gene pool of *O. sativa* for genetic improvement of drought tolerance (DT) of rice. Three elite lines, IR64, Teqing and a new plant type (NPT) were used as recurrent parents and 120 accessions of diverse origins were used as donors. A total of 375 BC_2F_2 populations were derived from crosses between the recurrent parents and donors and were subjected severe water stress under both lowland (at the reproductive stage) and upland conditions that killed the recurrent parents. A total of 4721 BC_2F_2 plants survived the stresses and were advanced to BC_2F_4 generation. The selected DT B_2C_4 introgression lines (ILs) were confirmed in progeny testing under the stress conditions and most ILs outperformed the parents for DT. Significant progresses have been made in large-scale DT gene/QTL discovery and development of DT rice cultivars by marker-aided gene/QTL pyramiding using these DT ILs. Currently, over 791 ILs selected from 40 BC populations were assayed using SSR markers and a highly efficient strategy was developed to identify genes/QTLs for DT using the ILs and linkage disequilibrium mapping. Two important results were obtained. First, many DT QTLs from a wide range of donors were detected. Second, DT QTLs tended to form highly associated groups. To verify these results, marker aided pyramiding of non-allelic genes/QTLs for developing superior DT rice cultivars has been initiated by making crosses between sister DT ILs. Several promising F_2 populations from these inter-ILs crosses were identified, from which a few hundred progeny with high level of DT were selected. These DT have been progeny tested under severe stress and genotyped by SSR markers. Preliminary analyses have confirmed all DT QTLs identified in the parental ILs and complex genetic networks consisting highly associated DT QTLs were discovered. Our results have led us to several conclusions. First, there is tremendous hidden diversity for DT in the primary gene pool of rice and most lines in the germplasm collection could be good donors for desirable alleles (traits) for DT regardless of their own performance in DT. Second, BC breeding combined with DNA markers are a powerful way to exploiting this diversity for simultaneous development of DT rice cultivars and discovery of genes/QTLs for DT. The implications of our results to plant breeding and the strategies using DT ILs for dissecting the genetic networks underlying DT and the development of superior DT rice cultivars by marker assisted gene/QTL pyramiding, will be discussed.

SPEAKER

INTEGRATED FUNCTIONAL GENOMICS IN BREEDING RICE FOR HIGH QUALITY AND ENRICHED NUTRITION

Vanavichit A. [1,2], *S. Sasoo* [1], *Amorn T.* [1], *S. Wanchana* [1], *W. Kamolsukyunyong* [2], *S. Ruengphayak* [1], *M. Siangliw* [2], *V. Ruanjaichol* [2], *T. Toojinda* [2] *and S. Tragoonrung* [2]

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Breeding high grain quality and nutritious rice for grain productivity is challenging. The completed genome sequencing and functional genomics have now empowered rice breeders with tools to create miracle rice strains. Positional cloning can become more efficient in the post-genomic era utilizing complete genomic sequence of the model rice. Grain aroma and submergence tolerance were two quantitative trait loci (QTL) being cloned efficiently using isogenic lines. Such a more multiplex QTLs as drought, isolated fragments carrying drought QTLs and blast QTLs were individually introgressed into the Thai Hom Mali Rice backbone using marker-assisted selection. Breeding high nutrition rice faces more challenges because of lack of genetic variation in cultivated rice. We set up a rice TILLING project using a nutritious and productive black rice to create useful genetic variation for molecular breeding and functional analysis. The irradiated mutant population consisted of 125,000 M1 seeds, M1-derived M2 seeds and two pooled genomic DNAs. Mutants have been identified using both forward and reverse genetic approaches. Because these black rice mutants can be readily cross-hybridized, new rice strains can be developed from these mutants. Under limited equipments available in developing countries, functional genomics can still be achieved and very useful for rice breeding if genetic materials and diversity are well prepared and chosen.

SPEAKER

Session 8
Plant-Microbe Interactions
Chair: Marc Orbach

Marc Orbach (U. Arizona) - A Genomics Approach to Pathogenicity: Saturation Insertional Mutagenesis in *Magnaporthe grisea*

Pamela Ronald (UC Davis): Signaling in the rice XA21-mediated defense response

Guo-Liang Wang (Ohio State University): Characterization of rice defense mutants using whole genome expression profiling

Jan Leach (Colorado State University): Approaches To Durable Resistance: Transfer Of Resistance Genes Between Cereal Species

Yulin Jia (USDA-ARS Stugartt, AK) - Molecular mechanisms of durable rice blast resistance

SPEAKER

A GENOMICS APPROACH TO PATHOGENICITY: SATURATION INSERTIONAL MUTAGENESIS IN *MAGNAPORTHE GRISEA*

Orbach,M.J. [1], *Tucker, S.L.*[1], *Betts, M.*[1], *Galadima N.* [1], *Soderlund,C.*[1], *Meng, Y.*[2], *Farman, M.L.* [2], *Li, L.* [3], *Xu, J.-R.* [3], *Donofrio, N.* [4], *Mitchell, T.K.* [4], *Dean, R.A.* [4]

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Magnaporthe grisea represents a model organism for the study of fungal pathogenicity and growth. We have created a collection of 50,000 DNA insertion lines in *M. grisea*, as part of an NSF-funded project to understand early events in the recognition and responses between this pathogen and rice. Our goal is to disrupt all genes encoded in the *M. grisea* genome in order to determine which genes are important for pathogenicity. To maximize coverage of the genome with insertions, different transformation constructs and transformation methods were used. DNA was introduced into *M. grisea* by both standard protoplast, and Agrobacterium tumefaciens-mediated transformation methods. All 50,000 insertion lines are being analyzed for defects in pathogenicity, metabolism and for alterations in morphology. Such a project necessitates development of high throughput methods for the generation and screening of putative transformants. In addition, a database has been created for recording all of the transformant data and making it accessible to the public. This presentation will focus on analysis of the pathogenicity mutants, along with advances in techniques for the genetic manipulation of this fungus and initial recovery of DNA insertions. In addition, initial microarray analysis of the infection process will be presented.

SPEAKER

SIGNALING IN THE RICE-XA21-MEDIATED DEFENSE RESPONSE

Ronald, P., Chern, M., Canlas P., Fitzgerald, H., Dardick, C., Richter, T., Lee, SW., Peng, Y., Han, SW., Ruan., DL.

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Components of innate immune systems in both plants and animals share many conserved features. Most notably, they sense the presence of pathogen-associated molecules (PAMs), which represent conserved molecular structures, and avirulence (Avr) factors that are strain specific molecules produced by phytopathogens. In animals, recognition of PAMs in extracellular compartments or at the cell surface is largely carried out by the toll like receptor (TLR) family that contain LRRs in the extracellular domain and a TIR intracellular domain (Werling and Jungi, 2003). Surprisingly, little is known about how plant hosts sense and respond to PAMs or Avr factors at the cell surface. The best-characterized examples are the Arabidopsis FLS2 receptor kinase that detects flagellin, a proteinasceous component of bacterial polar flagella, and the rice Xa21 receptor kinase that mediates recognition of *Xanthomonas oryzae* pv. *oryzae* (Xoo) strains expressing AvrXa21 activity. Given the importance of these receptors in innate immune recognition and host defense, we are interested in identifying the downstream signaling cascades and the PAMs that they recognize.Using the yeast two hybrid system with the XA21 kinase domain as a bait, we have identified several XA21 binding proteins (Xbs) that include Xb10, encoding a putative transcriptional regulator; and Xb15 encoding a PP2c phosphatase-like protein that are likely involved in transducing the XA21mediated defense response. Furthermore we have shown that an NPR1 rice homolog interacting protein, NRR, is a negative regulator of Xa21–mediated resistance.We have also identified eight Xoo genes, falling into three classes, which are required for AvrXa21 activity. raxA, raxB and raxC encode proteins with similarity to a membrane fusion protein, an ATP-binding cassette transporter and an outer membrane protein, respectively, of bacterial type I secretion systems (Goes Da Silva et al., 2004). The raxQ and raxP encoded proteins function in concert to produce phosphoadenosine phosphosulfate (PAPS), an active form of sulfate. The raxSt encoded protein shows similarity with mammalian and bacterial sulfotransferases that use PAPs as the sulfuryl donor (Shen et al., 2002; Goes Da Silva et al., 2004). Finally, two genes, RaxH and raxR, encode proteins with similarity to two-components regulatory systems and regulate raxSt expression (Burdman et al., 2004). Based on our results, we hypothesize that upon sensing of the plant environment, the AvrXa21 molecule is sulfated and then secreted by the RaxABC Type I secretion system making it available for race specific interactions with the rice receptor kinase XA21.

SPEAKER

References:

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CHARACTERIZATION OF RICE DEFENSE MUTANTS USING WHOLE GENOME EXPRESSION PROFILING

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Rice blast and bacterial blight are the two major diseases of rice causing significant yield loss in most rice-growing countries. Although many mutants exhibiting altered defense response to the two pathogens have been identified, very few are well categorized genetically. Taking advantage of the availability of whole genome rice oligoarrays, we started to dissect the rice defense pathways to the two major devastating pathogens using expression profiling. In our first round profiling, we selected four broad phenotypic classes, and within these classes, we selected 15 mutants and transgenic lines that show alterations in their interaction with one or more of these pathogens. Since the NSF-funded rice oligo array was not ready when the project started early this year, several pilot microarray hybridizations on four mutants were performed using Agilent's rice oligo arrays. Preliminary results indicate that the expression profiles of different mutants are significant different after pathogen inoculation. Using the newly released 20,230-element rice oligo array from the Ronald lab, expression patterns between four rice mutants and their wild type plants (two lesion mimic mutants and two NPR-related transgenic lines) will be compared after bacterial blight inoculation in early November. We also developed strategies for data acquisition, normalization and analysis using Gene Traffic 3.1 (Iobion Informatics) and Partek 6.0 (Partek) softwares. The mutant expression profiles from all the hybridization experiments will be made available on the project website www.ricedefensemutant.org for the public access.

SPEAKER

APPROACHES TO DURABLE RESISTANCE: TRANSFER OF RESISTANCE GENES BETWEEN CEREAL SPECIES

Leach,J.E., Zhao,B.Y., Lin,X., Poland,J., Trick,H., Hulbert,S.

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Kansas State University, Manhattan, KS 66506

Durable resistance to pests and pathogens is a long-sought goal of crop protection programs worldwide because it is considered cost-effective, environmentally sound, and promotes conservation of limited genetic resources. However, realizing durable resistance, which is controlled by both qualitative and quantitative traits, has been limited by the lack of understanding of its molecular basis. One approach to achieving durable resistance is to transfer non-host resistance (R) genes from one cereal, such as maize, into rice. However, whether or not non-host R genes would function in transfers between such distantly related grasses (e.g. rice and maize) was not known. We identified a non-host R gene, Rxo1, in maize that confers a hypersensitive response (HR) to the rice bacterial streak pathogen *Xanthomonas oryzae* pv. *oryzicola*. The gene mapped to the short arm of maize chromosome 6. An anonymous R gene-like sequence was used as a probe to identify a gene family that cosegregated with Rxo1 in several large mapping populations. The gene family members were subcloned, and a transient assay involving the *X. o*. pv. *oryzicola* effector gene avrRxo1 was used to identify the family member that functions as Rxo1. The identity of the Rxo1 gene was verified in stably transformed maize lines. Intriguingly, using both transient assays and stable transgenes we demonstrated that Rxo1 interacts with avrRxo1 and signals resistance in rice. These results demonstrate that Rxo1 functions in rice to condition resistance to *X. o.* pv. *oryzicola*.

SPEAKER

MOLECULAR MECHANISMS OF DURABLE RICE BLAST RESISTANCE

Jia, Y., Winston, E., Singh, P., Zhou, E., Wamishe, Y., Jia, M., and Correll, J.

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Durable resistance to the blast pathogen, Magnaporthe grisea, can be achieved by deploying a resistance gene that recognizes a virulence factor that is essential for the pathogen to cause disease. The Pi-ta gene in rice is effective in preventing the infection of M. grisea races containing the corresponding avirulence gene AVR-Pita. Pi-ta is a single copy gene located at the centromere of chromosome 12. Pi-ta encodes a predicted cytoplasmic protein with a centrally located nucleotide-binding site and a leucine rich domain at its carboxyl terminus. AVR-Pita is a metalloprotease located near the teleometric region of chromosome 3 of M. grisea. Pi-ta appears to recognize AVR-Pita inside the host cell triggering effective defense response. A survey of rice germplasm (in different rice production regions) has identified one resistant (Pi-ta) haplotype and three susceptible pi-ta haplotypes. Pi-ta confers resistance to the major US M. grisea pathotypes; the polymorphic regions of Pi-ta that distinguish resistant and susceptible alleles of Pi-ta were successfully used for the development of dominant and codominant markers for marker assisted Pi-ta incorporation. Survey of the pathogen population in the USA indicates that the deletion of the AVR-Pita allele in some “race -shift” isolates of M. grisea can defeat protection provided by Pi-ta. Race-shift isolates have been selected and recovered from the field. Whether such isolates can prevail and cause economic losses depends on the role of AVR-Pita in both pathogenesis and pathogen fitness in the environment. Progress on the development of molecular strategies to control rice blast disease will be presented.

SPEAKER

Workshop 2
Leveraging National and International Collaboration through IRFGC: current agenda and new initiatives
Chair: Hei Leung

Hei Leung: Overview of International Rice Functional Genomics Consortium: Setting goals, Steering Committee, USAID Linkage Program to strengthen IRFGC

Country / Insitute Updates
US – Jan Leach
Korea – Moo Young Eun
Japan – Hirohiko Hirochika
China – Qifa Chang
France – Emmanuel Guiderdoni
Australia - Liz Dennis
CIAT - Mathias Lorieux
Singapore - Ramachandran Srinivasan
Others

SPEAKER

Narayana Upadhyaya -Summary of Gene Tagging Workshop-the way forward

Andy Pereira – Phenotyping network

Perlegen Sciences / IRRI – SNP Project
Gene Expression Data

Open Discussion

LEVERAGING COLLABORATION THROUGH THE INTERNATIONAL RICE FUNCTIONAL GENOMICS CONSORTIUM

Hei Leung [1] *and Rod Wing* [2]

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[2]*Arizona Genomics Institute, University of Arizona, Tucson, Arizona 85721, USA*

The International Rice Functional Genomics Consortium (IRFGC) was formed in January 2003 to facilitate international collaboration to accelerate discovery of rice gene functions (http://www.iris.irri.org/IRFGC/). Since then, a number of activities and initiatives has demonstrated the benefits of leveraging support in working towards a common goal and provided incentives for expanding collaboration. Below is a brief update on these events to set the stage for further discussion at the Second Rice Functional Genomics Symposium in Tucson.

- A mutant paper entitled "Rice mutant resources for gene discovery" was published (Hirochika et al. 2004 Plant Molecular Biology 54: 325-334; http://www.kluweronline.com/issn/0167-4412). We are hopeful that this paper will encourage similar types of collective papers to promote international collaboration.
- Several laboratories under IRFGC are working together to develop a network project to systematically phenotype various mutant collections for conditional traits.
- USDA competitive grant program has supported projects that will advance IRFGC objectives.
    1. Systematic determination of the rice gene set (Cold Spring Harbor Laboratory)
    2. Use of oligo arrays to dissect rice defense response pathways (Ohio State University)
    3. TILLING resources for japonica and indica rice (University of Washington)

Furthermore, a new USDA project on applied genomics in rice has been initiated that will have active linkage with IRFGC.

- IRRI has invested funds from the USAID Linkage Program to support eight projects under IRFGC. This set of projects focuses on tolerance to biotic and abiotic stress with links to trait improvement and breeding activities. The contents of the projects and the investigators involved are shown in IRFGC website (http://www.iris.irri.org/irfgc/researchstrength.shtml)
- Several oligo-array platforms are now available for experimentation. Different partnerships have been or are being developed to maximize the utility of these platforms that could lead to further improvement and accessibility of the technology.
- An Indian rice functional genomics program has received support from the Department of Biotechnology of the Government of India. This national network is expected to collaborate closely with IRFGC participants.

The annual International Rice Functional Genomics Symposium provides an ideal forum to discuss matters relevant to IRFGC. An evening session on "National and International Collaboration: current agenda and new initiatives" has been scheduled at 8:00 to 10:00 pm, November 17. Tentative topics include: reports on national and international initiatives, setting realistic consortium goals, linking technology platforms, and more. All participants are encouraged to attend this session to contribute ideas to strengthen international collaboration.

SPEAKER

Session 9
Functional Genomics IV - Proteomics and Metabolomics
Chair: David Gang

Nijat Imin (Australian National University): Proteomic Analysis of Male Gametophyte Development and Its Response to Low Temperature Stress in Rice

Torsten Kleffmann (Swiss Federal Institute of Technology)- Organelle Proteomics of the Rice Etioplast to Chloroplast Development Reveals Insights into Regulatory Mechanisms of Plastid Biogenesis

Mark Lange (Washington State University) - Tools and approaches for surveying the metabolic capabilities of rice

Paul Haynes (University of Arizona) - Functional proteomics of orphan proteins in rice

Samuel S.M. Sun (Chinese University of Hong Kong) - Genomic study on the grain quality of hybrid rice

SPEAKER

PROTEOMIC ANALYSIS OF MALE GAMETOPHYTE DEVELOPMENT AND ITS RESPONSE TO LOW TEMPERATURE STRESS IN RICE

Imin,N, Kerim,T., Weinman, J.J. and Rolfe,B.G.

Australian National University and Australian Research Council Centre of Excellence for Integrative Legume Research

Rice (Oryza sativa L.) which was originated from the tropics and subtropics, is highly sensitive to cold temperature damage at the young microspore stage of pollen development. We used proteomic analysis to investigate the changing patterns of protein synthesis during pollen development in rice anthers and their responses to low temperature treatment that cause male sterility in rice. An anther proteome database of the proteins separated by two-dimensional gel
electrophoresis (2-DE) was established and publicly accessible at http://semele.anu.edu.au/2d/2d.html. Over 3,000 protein spots were detected over the pH range of 4-11. Of these, over 500 protein spots were analysed by MALDI-TOF MS (matrix-assisted laser desorption/ionisation time-of flight mass spectrometry), tandem MS and N-terminal sequencing and putative identities were assigned to more than 100 protein spots [1-2]. Then, we compared the anther proteome maps of different developmental stages and detected approximately 150 protein spots changed consistently during development. Of these, 44 proteins were identified including proteins are closely associated with sugar metabolism, cell elongation and cell expansion [3-4]. Furthermore, we detected 37 protein spots as differentially displayed after one, two and/or four days of cold temperature treatment in the cold sensitive cultivar Doongara. Of these, 13 proteins were identified. Proteomic analysis of late effect of early cold stress showed acceleration of partial protein degradation [5]. Some the significant findings of these studies will be highlighted.

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SPEAKER

ORGANELLE PROTEOMICS OF THE RICE ETIOPLAST TO CHLOROPLAST DEVELOPMENT REVEALS INSIGHTS INTO REGULATORY MECHANISMS OF PLASTID BIOGENESIS

Kleffmann,T., von Zychlinski,A., Russenberger,D., Gehrig,P., Sjölander,K., Baginsky,S., Gruissem,W.

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During plant development, tissue-specific programs control the differentiation of progenitor plastids into functionally specialized organelles, e.g. chloroplasts, etioplasts, amyloplasts or chromoplasts. To date, the knowledge about the mechanistic details underlying these differentiation processes is rather scarce. Here we report an extensive proteome analysis of the light induced development of the etioplast of dark-grown rice seedlings to the mature chloroplast.In organelle proteomics large scale datasets are difficult to interpret if potential contaminations cannot be excluded or the range of contamination cannot be estimated. We therefore established a new method to isolate plastids by practically 100% purity and a strategy to verify the purity by a comparative semi-quantitative analysis of characteristic marker proteins from other cell organelles. The protein complement of pure rice etioplasts was analysed by a complementary approach using LC-MS/MS shotgun protein identification and 2-D PAGE-coupled MALDI TOF-TOF-MS. In a time course experiment the etioplast to chloroplast conversion was investigated by differentially displaying the protein profiles at 2, 4, 8 and 24 hours of de-etiolation. Both approaches together resulted in more than 430 different protein identifications from the etioplast. Most of the identified proteins function in an etioplast characteristic heterotrophic metabolism and gene expression. A systematic comparison of the here identified etioplast protein complement with those identified from plastids of different plant species reveals no rice specific proteins, but proteins which may have a significant function in the dark grown etioplast. These are mainly hypothetical proteins or proteins function in gene expression. Computational structure analysis of proteins without an annotated function results in potential novel protein functions for the plastid compartment, such as a membrane bound site 2 metalloprotease. Site 2 metalloproteases are involved in the control of gene expression in prokaryotes as well as in eukaryotes by a proteolytic activation of transcription factors.

The quantitative proteome analysis reported here provides extensive insights into the dynamics of protein expression and post translational modifications at the early stage of etioplast to chloroplast development. Whereas the expression of proteins involved in housekeeping pathways such as Calvin cycle, pentose phosphate pathway, tetrapyrrol biosynthesis and Shikimate pathway are kept constant during the first four hours after illumination, different chlorophyll-a,b-binding proteins are early up-regulated. Immediately at the onset of illumination, the phosphorylation status of plastid RNA-binding proteins (RNPs) increases slightly. Phosphorylation of RNPs is thought to alter their RNA-binding properties, which is important for RNA stability and regulation of gene expression. The novel protein functions in the plastid together with the differential phosphorylation of RNPs will be discussed in the context of new mechanisms that are potentially involved in the regulation of plastid gene expression and differentiation.

SPEAKER

TOOLS AND APPROACHES FOR SURVEYING THE METABOLIC CAPABILITIES OF RICE

Lange, B.M., Presting, G.G.

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Functional genomics, the science of deciphering DNA sequence structure, variation, and function, is expected to become the engine driving the discovery of traits and to help solve intractable problems in crop production. The recent completion of the rice (Oryza sativa) genome sequence represents an enormous pool of information for rice improvement through marker-aided selection or genetic engineering. Yet, a full exploitation of this wealth of information will not be possible until we understand the biological functions encoded by the sequenced DNA. A genome-wide experimental approach will be instrumental in dissecting metabolic pathways important for increasing rice productivity and nutritional content. In this presentation, progress toward the elucidation of specific metabolic pathways linked to key quality traits in rice will be highlighted and a framework for the implementation of a knowledge database will be suggested.

SPEAKER

FUNCTIONAL PROTEOMICS OF ORPHAN PROTEINS IN RICE

Tim Radabaugh and Paul A. Haynes

Department of Biochemistry and Molecular Biophysics, Bio5 Institute for Collaborative Bioresearch, The University of Arizona, Tucson AZ 85721.

Our goal is to perform functional proteomic characterization of orphan proteins in Oryza sativa (Rice), with the objective of creating a resource for the plant biology research community. We will first undertake an exhaustive proteomic survey of leaf, root, seed and callus tissue from rice, and extract from this data a subset of expressed proteins consisting of all those that are identified yet have no known function or homology to known proteins. These orphan proteins will then be expressed as TAP-tagged transformants in rice callus tissue, and we will use mass spectrometric techniques to identify the binding partners of each orphan in immunoprecipitation pull-down experiments. This data will be used to infer an initial protein function on the basis of protein-protein interactions identified. Additional functional information will be produced from quantitative RT-PCR experiments performed on each identified orphan protein, with gene expression levels examined under the imposition of several different abiotic stress conditions. All of this data will be integrated with existing rice genome database resources, and made publicly available for the benefit of the greater plant biology research community. We expect this project to serve as a starting point for many future research programs based on further detailed characterization of the orphan proteins for which we will provide an initial functional annotation.
We have begun collecting data for our proteomics analysis, specifically from SDS-PAGE – nanoLC-MS/MS of rice leaf, root and seed. Protein extracts were prepared and run on SDS-PAGE gels stained with silver and destained prior to in-gel digestion. Each lane was cut into 32 pieces and in-gel digested with trypsin. Each tryptic peptide digest was analyzed by nanoLC-MS/MS and resulting MS/MS spectra were Sequest searched against a combined plant protein database including copies of the translated rice and Arabidopsis genome sequences. Applying high stringency results filtering, we identified 766 proteins in leaf, 446 proteins in root and 757 proteins in seed. The combined non-redundant total of proteins identified in these three experiments was 1422 distinct proteins, which included a small number of orphan proteins. In our experience it is a relatively simple matter to produce large numbers of unique protein identifications from a given tissue sample in the initial experiments performed. These generally represent mostly the most abundant proteins in the cell. It is, however, far more difficult, and requires a lot more work, to identify a large number of additional proteins from the same material. These additional identifications represent the next level down in terms of abundance, and we expect these will include significantly more orphan proteins.

SPEAKER

GENOMIC STUDY ON THE GRAIN QUALITY OF HYBRID RICE

Sun,S.S.M., Long,X.H., Liu, Q.Q., Zhang,J.J. and Leung, K.K.

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Rice is the staple food for half of the world population. As mature rice seeds are predominantly composed of starch and protein (80-90% and 8-10%, respectively, as dry matter), during grain filling, the biosynthesis and accumulation of starch and protein in the seeds plays a determining role in the grain quality and yield of rice. Hybrid rice was first developed in China and released in 1975. It has a 20% yield advantage over the best conventional rice varieties. The super-hybrid rice currently under development in China aims at another 15% yield increase over the existing hybrid rice. Currently about 50% of the Chinese rice fields is growing hybrid rice varieties. Although hybrid rice has yield advantage, its grain quality, especially the cooking and eating quality, however, is generally not high. The composition/property of starch and to a lesser extent, storage proteins, is a major factor influencing grain quality of rice. We are interested in the grain quality of hybrid rice and have been profiling the expression of genes involved in starch, reserved proteins, and lysine biosynthesis and metabolism during rice grain filling. Recently we have initiated the analysis of the amyloplast proteome of the developing seeds of selected hybrid rice lines. Proteins from the amyloplasts were separated by 2-(and 1-) dimensional gel electrophoresis and analyzed by PMF and MS/MS techniques. We have identified over 150 proteins from the amyloplast preparations at this stage and carried out some initial analysis. This and other related works on the hybrid rice grain quality will be reported. (This research is supported by an AoE grant from UGC HKSAR and a RAC special fund from CUHK).

SPEAKER

Session 10
The Future of Rice Genomics Research
Chair: Rod A. Wing

Richard Nelson (Noble Foundation)- Virus-Induced Gene Silencing in Rice for Gene Function Determination

Blake Meyers (University of Delware)- Deep transcriptional profiling of rice using MPSS

Vicki Chandler (University of Arizona) - Epigenetic control of gene expression

Joseph Ecker (Salk Institute) - Systematic genome-wide screens in Arabidopsis

Rod Wing (University of Arizona): Closing Remarks and Announcement of Location and Chair of Next Meeting

SPEAKER

VIRUS-INDUCED GENE SILENCING IN RICE FOR GENE FUNCTION DETERMINATION

Nelson, R. S., Ding, X.S., Ballard, K., Chaluvadi, S.

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Virus-induced gene silencing (VIGS) is a powerful technique for the study of gene function through transcript knock-out. The technique is based on the use of a virus vector to over-express RNA derived from a host gene, which is then recognized as aberrant and targeted for sequence-specific destruction together with the host transcript. The technique has been used successfully in reverse and forward genetic screens to determine the function of genes in dicotyledonous plants. To date VIGS has not been used to study gene function in rice because no rice-infecting virus amenable to this procedure was known. We have identified a strain of Brome mosaic virus (BMV), F-BMV, which infects multiple monocot species, including rice. The virus was cloned and altered to serve as a vector during VIGS studies. Expression of host genes such as phytoene desaturase and actin by the virus vector in rice and other monocot hosts led to unique visual phenotypes consistent with those expected for gene silencing and to the destruction of the specific host transcript in the affected tissue. The status of VIGS for use in dicots and monocots and the methods being investigated to optimize the technique for use in plants, with emphasis on rice, will be presented.

SPEAKER

DEEP TRANSCRIPTIONAL PROFILING OF RICE USING MPSS

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We are using the transcriptional profiling technology called massively parallel signature sequencing (MPSS) to characterize the diversity and abundance of rice transcripts. Like SAGE (Serial Analysis of Gene Expression), MPSS produces short sequence tags ("signatures"; 17 or 20 bp) produced from the most 3' DpnII site (GATC) of each the mRNA molecule. The relative abundance of these tags in a given library represents a quantitative estimate of expression of that gene. This is an "open" gene expression platform that can define a nearly-complete set of transcripts in a given tissue. We are using MPSS to quantify the expression of transcripts in wildtype tissues, in fungal- or bacterial-infected rice tissues, and in indica and japonica parents and hybrids. With these data, we are creating query and analysis tools to facilitate public use of and access to rice MPSS data. Our web interface displays the abundance and chromosomal locations of rice MPSS signatures. This project will develop resources for annotation of the rice genome by sampling more than 75,000,000 transcripts while also precisely defining the expression levels of those transcripts in diverse tissues and treatments. I will discuss our recent progress in this project, as well as the implications of a novel MPSS-based method for the identification and measurement of small RNAs that we have been applying to plants.

SPEAKER

EPIGENETIC REGULATION OF GENE EXPRESSION

Vicki Chandler

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Epigenetics is an exciting and rapidly evolving field that impacts basic science, agriculture and clinical medicine. Epigenetic regulation refers to heritable control mechanisms superimposed on the DNA sequence. We investigate the control of gene expression in plants using the regulated expression of the genes required for the biosynthesis of the purple anthocyanin pigments in maize. Anthocyanin pigments have provided excellent visual and molecular markers to geneticists who used these genes to discover a number of important epigenetic phenomena in plants such as the cycling of transposons between silent and active states, transgene silencing and paramutation. Paramutation is an interaction between alleles that causes a directed, heritable alteration in the expression of one allele. In my laboratory we are using a combination of genetic and molecular approaches to determine the underlying mechanisms associated with epigenetic control of gene expression. In my talk I will describe the various epigenetic phenomena, discuss models for how chromatin-level control can produce heritable changes in gene expression, and discuss evolutionary implications of this type of gene regulation. Current ideas are that these mechanisms may have evolved to regulate gene dosage, maintain genome integrity, and defend against viruses and invasive DNA.

SPEAKER

SYSTEMATIC GENOME-WIDE SCREENS IN ARABIDOPSIS

J.R.Ecker [1] H.Chen [1], A.Sundarasen [1], J.Borevitz [1], R.Cheuk [1], C.J.Kim [1], J.Lim [1], J.J.Ecker [1], T.Mockler [1], P. Shinn [1], C.Meyers [1], M..Karnes [1], E.Koesema [1], Y.Ansari [1], N.Choy [1], K.Yamada [2], J.M. Dale [2], H.C. Wu [2], S.X. Liu [2], H. Sakano [2], G. Yu [2], C.H.Chang [2], P.Pham [2], V.W.Hsuan [2], H.L. Quach [2], P.X.Jiang [2], J.M.Lee [2], M.Toriumi [2], M.M.H.Chan [2], C.C.Tang [2], C.S. Onodera [2], J.M. Deng [2], A.D. Goldsmith [2], M.Vaysberg [2], E.K.Wallender [2], C.Wong [2], Y.Yamamura [2], S.Yuan [2], J.Banh [2], F.Banno [2], C.J. Palm [3], A.M.Southwick [3], T.Jones [3], M.Nguyen [3], G.Karlin-Newmann [3], B.Lam [3], M. Miranda [3], M.Gurjal [3], N.F.Hansen [3], L.Bowser [3], T.Wu [3], M.Tripp 3, R.Tamse [3], M.Seki [4], T.Sakurai [4], M.Satou [4], K.Akiyama [4], P.Carninci [4], A.Enju [4], Y.Hayashizaki [4], K.Iida [4], J.Ishida [4], T.Arakawa [4], J.Kawai [4], A.Kamiya [4], M.Nakajima [4], M.Narusaka [4], S.Chan[5], X.Zhang[5], K Shinozaki [4], R.W.Davis [3], A.Theologis [2], S.Jacobsen[5]

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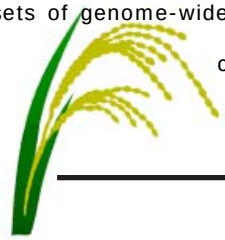
SPEAKER

Complete genome sequences are now available for a wide variety of organisms and many more are on the way. In order to carryout functional analysis in these organisms, accurate determination of gene structures and complete gene inventories will be essential. Computational gene prediction methods are improving but alone are inadequate for new gene discovery and accurate annotation of genomes, in particular for certain gene classes such as non-coding RNA genes. New approaches are required to identify the entire complement of transcription units (protein coding and non-coding), and their associated regulatory elements (e.g. TF/chromatin binding locations and sites of DNA methylation). We are pursuing empirical approaches to decode this information using the genome sequence of the reference plant Arabidopsis, enabling more rapid assessment of the biological functions of the ~30,000 predicted genes. Unbiased mapping of the transcription units is being carried out using third-generation Affymetrix whole genome tiling array (WGA) technology. The high-resolution transcription unit location information is being used to guide the construction of a complete, expression-ready, gene inventory- "the plant ORFeome". We have also begun to utilize WGAs as a "universal" data-gathering platform for capturing a variety of types of genome-scale information, including the chromosomal locations of DNA methylation sites (and identification of the methylases that target these sites), chromatin/transcription factor binding sites and for discovery of genome-wide allelic variations among geographically isolated Arabidopsis accessions. When coupled with transcriptome mapping data, these unbiased sets of genome-wide regulatory information will begin to allow the construction of an integrated set of cellular/molecular connectivity maps for Arabidopsis.



SEARCH FOR OSGI (A RICE ORTHOLOGUE OF THE ARABIDOPSIS GI) INTERACTING PROTEINS BY TANDEM AFFINITY PURIFICATION (TAP) SYSTEM

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OsGI is the one of the genes involved in the photoperiodic control of flowering in rice. OsGI encodes a putative nuclear localization protein, however its biochemical function is unknown.

Here we try to understand how the molecular mechanism of the photoperiodic control of flowering is regulated in rice, a short-day (SD) plant. To understand biochemical function of OsGI, we attempt to identify members of the OsGI protein complex under native conditions by using the TAP system. The TAP system showed the rapid purification of complex without prior knowledge of the complex composition, activity, or function.

In order to isolate the OsGI complex in rice, we produced TAP tag constructs, N-terminal TAP (SGN) and C-terminal TAP (SGC) and transformed them into rice. OsGI and C-terminal TAP tagged OsGI were detected in the cytosolic fraction by western blotting analysis with OsGI specific antibody. We are now purifying proteins interacting with OsGI from the cultured cell (SGC lines) by using the TAP system. Results will be shown.

POSTER

RICE FUNCTIONAL GENOMICS RESOURCES FROM JAPAN

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The Program for Rice Genome Research (PRGR) in Japan, through various projects in rice genomics, has generated valuable resources, which could serve as indispensable tools in understanding the structure and function of the rice genome. These resources have been made available to the scientific community through the Rice Genome Resource Center (RGRC) to enable rapid progress in research that will lead to thorough understanding of the rice plant. The genetic stocks currently available for distribution include the rice full-length cDNA clones, Tos17 insertion mutant lines and plant materials for genetic analysis. As the next trend in rice genome research will focus on determining the function of about 40-60,000 genes predicted in the genome as well as in applying various genomics tools in rice breeding, an unlimited access to rice DNA and seed stocks will provide a broad community of scientists with the necessary materials for formulating new concepts, developing innovative researches and making new scientific discoveries in rice genomics.

POSTER

Poster # 87

A STRESS RESPONSIVE MAP KINASE MEDIATES ABSCISIC ACID AND ETHYLENE SIGNAL INTERACTION IN RICE PLANTS

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Mitogen activated protein kinases (MAPKs) mediate signal integration as points of convergence and divergence through their involvement in a plethora of biological processes, including plant growth and development as well as abiotic and biotic stress responses. As a component of an integrated signaling network, MAP kinase cascades transduce the perception of environmental cues to the intracellular milieu. During abiotic and biotic defense responses plant cells amplify the initial perception event through the release of secondary signaling molecules, such as abscisic acid (ABA) and ethylene (ET). ABA and ET are important signaling molecules involved in abiotic and biotic stress responses, however little is known about the molecular mechanism underlying their signal transduction and interaction in rice. Recently, an ABA-inducible rice mitogen activated protein kinase (OsMAPK5) was shown to inversely modulate disease resistance and abiotic stress tolerance. In this study we have determined the role of OsMAPK5 in mediating ABA and ET signal interactions during rice defense responses. In rice ABA and ET appears to act antagonistically during defense responses. Exogenous application of ET was reported to enhance disease resistance in rice. Interestingly, treatment of rice plants with ABA decreased endogenous ET levels and reduced disease resistance to the rice blast fungus, Magnaporthe grisea. Transgenic analysis demonstrated that suppression of OsMAPK5 expression via RNA interference resulted in decreased sensitivity of seed to ABA during seed germination, elevated levels of endogenous ET, activation of defense genes and enhancement of disease resistance. Treatment of these transgenic plants with ABA leads to a more drastic decrease of ET levels than wildtype plants, suggesting that the ABA-inducible OsMAPK5 serves in some capacity to regulate ET levels during defense responses. By contrast, the endogenous ET levels and the susceptibility to M. grisea in OsMAPK5 overexpression transgenics were constant and similar to wildtype, respectively; these plants are however more resistant to abiotic stress. All this evidence taken together suggests OsMAPK5 is a key signaling component mediating the antagonistic interaction of ABA and ET pathways during biotic and abiotic stress responses.

POSTER

Poster # 88

FUNCTIONAL GENOMICS OF RICE SUSCEPTIBILITY TO BACTERIAL DISEASES

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Bacterial blight and bacterial leaf streak of rice are economically important diseases in many rice-growing regions of the world and are representative of the two major types of disease caused by Gram-negative pathogens in plants. Blight is caused by Xanthomonas oryzae pathovar oryzae, which enters through wounds or water pores (hydathodes) in the leaf and invades the vascular tissue. Leaf streak is caused by Xanthomonas oryzae pv. oryzicola, which typically enters through stomata and colonizes the intercellular spaces of the leaf photosynthetic tissue. Because the bacteria are members of the same species (they are greater than 90% similar by DNA hybridization studies) and the host is a model cereal, together these diseases constitute a uniquely valuable model system for understanding the pathogen and host traits that create opportunities for microbes to exploit different plant tissues. An understanding of the pathogen and host traits that create opportunities for microbes to exploit different plant tissues will advance our understanding of plant biology and support innovative efforts to alter plant susceptibility or block pathogen virulence. We are working toward identifying rice genes that affect susceptibility to the two pathogens by screening for mutants with altered susceptibility and by comparing global transcription profiles of plants infected with one or the other pathogen. We are also characterizing important genetic differences between the pathogens using a comparative genomics approach combined with mutagenesis, heterologous expression, and reporter gene assays. Our poster provides details on these ongoing efforts and results to date.

POSTER

Poster # 89

BIOINFORMATICS FOR RICE FUNCTIONAL GENOMICS AT IRRI

Bruskiewich, R.M. [1,2,3,4], *Portugal, A.M.* [2], *Cosico, A.B.*[2], *Despacio-Reyes G.*[2], *Ulat, V.J.M.*[2], *Pimentel, C.I.*[2], *Ulat, M.T.*[2], *Mansueto, L.*[2,3], *Gregorio, S.*[2,4], *Eusebio, W.*[2], *Mendoza, M.J.*[2], *Sallan, M.A.B.*[2], *Sison, L.*[2],. *Herrera, R.Q.*[2], *Constantino, W.V.E.*[2], *Mauleon, R.*[2,4], *Sylvester, A.G.* [2,4], *Sackville-Hamilton, R.*[2], *Leung, H.*[2], *McNally, K.L.*[2], *Metz, T.*[2], *and McLaren, C.G.*[2].

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[3] *University of the Philippines at Diliman, and*
[4] *University of the Philippines at Los Baños*

The International Rice Research Institute has an active program of rice functional genomics that includes a significant bioinformatics component housed in the Institute's Biometrics and Bioinformatics Unit.The focal of these activities is the International Rice Information System (IRIS, www.iris.irri.org) is the rice implementation of the open source International Crop Information System (ICIS, www.icis.cgiar.org) project, a comprehensive platform for the management and integration of germplasm (including genetic resources, breeding and genetic stocks), genotype and phenotype information for any crop, under development for many years through a global partnership of crop scientists and informatics developers.
Our presentation will cover the various functional genomics components of IRIS including:

- The IR64 rice mutant database.
- New IRIS facilities for the capture, representation, analysis, integration and publication of high-throughput crop genomic and molecular variation (genotype) data, with an emphasis on ontology-driven annotation of data.
- A novel Java language port of the ICIS system providing several significant functional enhancements over earlier systems, including novel specialist views to the system in the form of newly developed stand-alone applications and an enhanced, next generation web portal.
- State-of-art internet protocols and technology now embedded into the system to support a globally distributed community of databases.

Our presentation will also report on the general application of ICIS functional genomics technology to other crop species within the CGIAR-hosted Generation Challenge Program (www.generationcp.org).

POSTER

Poster # 90

MARKER-ASSISTED SELECTION OF A GENE RESISTANT TO BROWN PLANTHOPPER BPH1 IN RICE (*ORYZA SATIVA*)

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Brown planthoppers, Nilaparvata lugens are a major insect pest of rice throughout South and Southeast Asia. They do plants much damage and cause considerable loss of rice production. The pest insect has been controlled mainly with insecticides and resistant varieties. The application of resistant varieties is environmentally safer and economically sounder than insecticides, even though the resistance is broken down by occurrence of new biotypes of the pest. Having been developed so far, many varieties harbor Bph1, bph2, or both as resistance sources. We have developed four specific DNA markers (called 'pBPH9', 'pBPH19', 'pBPH20' and 'pBPH21') to be detectable Bph1 through PCR. They were made based on the sequence of a clone of CUGI OSJNBa library 0040P10. In case of pBPH9 the PCR products amplified from 37 varieties were classified into two types (536 bases in resistant varieties and 773 bases in susceptible varieties). Such tendency was presented in the rest three primer sets. Therefore it was concluded that the markers would be very useful in the selection of resistant plants with Bph1. However a exception was observed in a resistant variety 'Namyeong', reported to be with Bph1. Its reaction to brown planthoppers did not accord with the type of PCR product.

POSTER

Poster # 91

INVESTIGATING SECONDARY METABOLISM IN RICE USING AN INTEGRATED FUNCTIONAL GENOMIC APPROACH

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Plants collectively make an enormous array of small molecular weight compounds, known as secondary metabolites. These compounds play important roles in the physiology and ecology of the plants that produce them, particularly as defense elements against insects and pathogens. Sequence analysis of the rice genome reveals a number of protein families that are putatively involved in secondary metabolism. These include terpene synthase, chalcone synthase, cytochrome P450, and substitution enzymes such as methyl transferase and acyltransferase. The presence of large protein families of secondary metabolism in the rice genome suggests that rice possesses the capability of producing a large number of secondary metabolites, most of which are probably unknown to the phytochemists. Elucidating the functions of these genes is an integral part of understanding the biology of rice. An integrated functional genomic approach, which combines bioinformatics, expression profiling, metabolic profiling, and large-scale in vitro biochemical assays, is being employed in our laboratory to investigate the functions of the genes of secondary metabolism in rice. The knowledge gained from this study is expected to provide novel tools for plant metabolic engineering, and also shed important new light on the evolution of secondary metabolism.

POSTER

NRR, A NEGATIVE REGULATOR OF DISEASE RESISTANCE IN RICE THAT INTERACTS WITH ARABIDOPSIS NPR1 AND RICE NH1

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Arabidopsis NPR1/NIM1 is a key regulator of systemic acquired resistance (SAR), which confers lasting broad-spectrum resistance. Over-expression of Arabidopsis NPR1 or the rice NPR1 homologue 1 (NH1) in rice results in enhanced resistance to the pathogen Xanthomonas oryzae pv. oryzae (Xoo), suggesting the presence of a related defense pathway in rice. We investigated this pathway in rice by identifying proteins that interact with NH1. We will report the isolation and characterization of a rice cDNA encoding a novel protein, named NRR (for Negative Regulator of Resistance), which negatively regulates resistance to Xoo when over-expressed in rice. NRR interacts with NPR1 in the NPR1-interacting domain (NI25) consisting of 25 amino acids. NRR also interacts with NH1; however, NI25 was not sufficient for a strong interaction, indicating a difference between the rice and Arabidopsis proteins. When constitutively over-expressed in rice, NRR affected basal resistance, age-related resistance and Xa21-mediated resistance, causing enhanced susceptibility to Xoo. This phenotype was correlated with elevated NRR mRNA and protein levels and increased Xoo growth. Over-expression of NRR suppressed the induction of defense-related genes. NRR:GFP protein was localized to the nucleus, indicating that NRR may act directly to suppress activation of defense genes. NRR is the first gene demonstrated to compromise Xa21-mediated resistance, indicating cross-talk or overlap between NH1- and Xa21-mediated pathways.

POSTER

Poster # 93

A WHOLE GENOME *MAGNAPORTHE GRISEA* MICROARRAY, VERSION 2.0.

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Magnaporthe grisea, the fungus that causes rice blast disease, is one of the main pathological threats to food supplies worldwide. Annually, enough rice is lost to this disease to feed 60 million people per year. Strains of this fungus attack other cereals, including wheat and barley. The disease is also a serious problem of turf grass. Moreover, few fungal plant pathogens rival rice blast for the sophistication of molecular and classical genetic tools that have been developed, nor the breadth and the depth of the rice blast research community. The 40 Mb genome is contained within 7 chromosomes for which extensive genetic and physical maps are available. Rice blast is a compelling experimental system for elucidating numerous aspects of host-parasite interactions, including infection related development, cultivar and species specificity and associated signaling pathways. Magnaporthe grisea strain 70-15 has recently been completely sequenced (6x coverage via whole genome shotgun sequencing) and annotated. The current version of the genome contains about 14,000 genes and has been used as a template to design a whole Magnaporthe genome array based on Agilent Technologies in situ 60 mer oligonucleotide probes. This design has recently been modified to reflect changes in annotation of the *Magnaporthe grisea* genome, and, in particular the recent availability of both the rice full length cDNA and the rice genome databases. This newly released array contains 15170 *Magnaporthe grisea* features derived from the Broad Institute genome sequence (v4.0),which have been re-annotated by NCSU. 12,508 of these features are common to the original array. In contrast, there has been an extensive re-design of the rice content of this array. There are 6325 rice features of which 4878 are new and 1447 are retained form the original array design. Some initial gene expression experiments will be described showing global changes in transcript profiles between *Magnaporthe grisea* grown under two differential vegetative states: Mycelia v Appressoria (infectious cells). In addition some preliminary experiments will be described showing the detection of Magnaporthe grisea transcripts in infected rice leaf tissue.

POSTER

Poster # 94

THE OSSLT1 GENE OF RICE IS POSTTRANSLATIONALLY REGULATED AND FUNCTIONS AS A CHAPERONE UNDER STRESS CONDITIONS

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A large number of genes that are conserved between monocots and dicots are still uncharacterized. In an effort to uncover the biochemical functions of novel stress-related genes, the japonica rice homolog of the plant-specific sodium and lithium tolerance (OsSLT1) gene encoded by a 1,563-bp intronless open reading frame on chromosome-1 was characterized. The N-terminus of the 56-kDa OsSLT1 contains a glycine-rich region and a carboxypeptidase site, while the C-terminus contains a chaperone-like domain similar to the alpha-crystallin-type small heat shock proteins with C-terminal extension. Northern blot analysis showed that the full-length mRNA is transcribed at high levels in seedlings under normal condition and also when exposed to chilling, dehydration and high salt conditions. Immunodetection indicated that the function of OsSLT1 polypeptide during stress is defined at least in part by the chaperone-like domain and regulated by differential posttranslational cleavage of the N-terminal and C-terminal regions. Comparative biochemical analysis indicated that cleavage of the C-terminal extension of the chaperone domain enhances chaperone activity based on the ability of the truncated protein to prevent the heat-induced inactivation of citrate synthase and its higher level of protease activity. The results presented in this study provide the evidence that posttranslational modification of the OsSLT1 gene product is a necessary step for the protein to perform its various functions, which includes the prevention of massive protein denaturation and aggregation that often occur in plant cells under low temperature, dehydration and osmotic stress conditions.

POSTER

Poster # 95

THE GENERATION AND ANALYSIS OF A LARGE POPULATION OF INSERTIONAL MUTATIONS INDUCED BY AC/DS-MEDIATED GENE TAGGING SYSTEM IN RICE

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This project is performed to develop a large population of insertional mutations, and to construct databases of molecular information on Ds insertion sites. Ultimate goals are to supply genetic materials and information essential for functional analysis of rice genes and for breeding using agronomically important genes. Two strategies have been employed to generate the large scale of insertional mutagenized population; 1) genetic crosses between Ac and Ds lines and 2) plant regeneration from seeds of Ac Ds. Over 70% of regenerated plants generally carried independent Ds elements. An exceptionally high proportion of independent transposants in the regenerated population does not require selection for transposed Ds and continual monitoring of Ac/Ds activities may not necessarily be required. For molecular identification of Ds insertion sites, genomic DNA was extracted from leaf tissues of F2 and T1 (or T2) plants. Ds-flanking DNA has been amplified via iPCR and TAIL-PCR and been directly sequenced. So far, over 60,000 lines have been generated and over 8,000 Ds loci have been identified by sequence analysis. Database of molecular information on Ds insertion sites has been constructed, and will be updated soon and opened to public at http://www.niab.go.kr. The acquisition of genomic DNA and Ds flanking sequence of tagging lines and the development of databases of tagging lines will be a very pivotal step to perform functional study of whole rice genes.

POSTER

GENETIC MAPPING OF MICROSATELLITE (SSR) MARKERS LINKED TO THE RICE PI-I BLAST RESISTANCE GENE

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Rice blast is a major fungus disease threat to rice production throughout the world. Genes conferring resistance to blast are a valuable and environmentally-friendly means to minimize crop losses from this serious disease. The Pi-i blast resistance gene has been utilized worldwide as a source of disease resistance, and has been indirectly mapped onto rice chromosome 9. Inukai et al. (1994) found that Pi-i mapped 6.2 cM from Pi-3; Jeon et al. (2003) mapped Pi-5 onto rice chromosome 9 and also reported that the Pi-3 and Pi-5 have identical genomic regions; and Yi et al. (2004) reported the development of unique dominant markers for the Pi-5 gene that were only present in advanced monogenic rice lines carrying the Pi-i, Pi-3, and Pi-5 genes and in cultivars historically recognized as carrying the Pi-i gene. Herein, we report the direct linkage analysis of the Pi-i gene in USA germplasm using co-dominant microsatellite (SSR) markers mapping near the Pi-5 locus identified by Jeon et al. Pi-i mapping was analyzed in two genetic crosses made between the cultivar L-205, carrying the Pi-i gene, and two breeding lines lacking Pi-i. Progeny from these crosses segregating for the Pi-i gene were screened for resistance to blast race IH-1. Results indicate that the Pi-i blast resistance factor conferring resistance to race IH-1 maps to the same region as the Pi-5 gene, supporting the findings of Jeon et al. and Yi et al. The presence of several marker haplotypes among cultivars reported to have the Pi-i gene and IH-1 resistance indicates that this gene region is highly variable and that numerous resistance alleles can be found at the Pi-i/Pi-3/Pi-5 locus. The microsatellite markers we identified to be closely linked to the Pi-i gene can readily be used for marker assisted selection of this blast resistance gene.

POSTER

Poster # 97

EVOLUTIONARY HISTORY OF ORYZA SATIVA LTR RETROTRANSPOSONS: A PRELIMINARY SURVEY OF THE RICE GENOME SEQUENCES

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LTR Retrotransposons transpose through reverse transcription of a RNA intermediate and are ubiquitous components of all eukaryotic genomes thus far examined. Plant genomes, in particular, have been found to be comprised of a remarkably high number of LTR retrotransposons. There is a significant body of direct and indirect evidence that LTR retrotransposons have contributed to gene and genome evolution in plants. To explore the evolutionary history of long terminal repeat (LTR) retrotransposons and their impact on the genome of Oryza sativa, we have extended an earlier computer-based survey to include all identifiable full-length, fragmented and solo LTR elements in the rice genome database as of April 2002. A total of 1,219 retroelement sequences were identified, including 217 full-length elements, 822 fragmented elements, and 180 solo LTRs. In order to gain insight into the chromosomal distribution of LTR-retrotransposons in the rice genome, a detailed examination of LTR-retrotransposon sequences on Chromosome 10 was carried out. An average of 22.3 LTRretrotransposons per Mb was detected in Chromosome 10. Our invesatgation suggested that gypsy-like elements were found to be > 4 × more abundant than copia-like elements. Eleven of the thirty-eight investigated LTR-retrotransposon families displayed significant subfamily structure. We estimate that at least 46.5% of LTR-retrotransposons in the rice genome are older than the age of the species (< 680,000 years). LTR-retrotransposons present in the rice genome range in age from those just recently inserted up to nearly 10 million years old. Approximately 20% of LTR retrotransposon sequences lie within putative genes. The distribution of elements across chromosome 10 is non-random with the highest density (48 elements per Mb) being present in the pericentric region.

POSTER

Poster # 98

ANALYSIS OF REPETITIVE DNA IN CHROMOSOMES 1, 3 AND 10 OF ORYZA SATIVA CV. NIPPONBARE AND BAC END SEQUENCES FROM THREE WILD RELATIVES

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About 40% of the rice (*Oryza sativa L.*) genome is comprised of repetitive DNA. The presence of such large amounts of repetitive DNA in the genome often pose problems in sequence assembly and thus render genome sequencing and analysis difficult. Moreover, the probable effects of these rapidly evolving elements on gene duplication and regulation of gene expression further necessitate the study of repetitive DNA in the context of genomic diversity and plant evolution. An inquiry into the distribution, nature and the basis of interspersion of repeats in the rice genome can shed some light on the relationship and evolution of different rice genomes as well as the origin of cultivated rice. We used the TIGR Oryza repeat database to identify and map repetitive sequences on chromosomes 1, 3 and 10 of Oryza sativa cv. Nipponbare. Best global alignment at base identity 95% shows that in these three chromosomes, the retroelements constitute a major proportion of repeats followed by transposons and mites. Unclassified repeats also occupied a considerable fraction of the total repetitive DNA. In order to study the evolutionary significance of these sequences, similar repeat analyses were also done using the BAC end sequences of three wild relatives of rice viz., *Oryza rufipogon* (AA genome), *Oryza nivara* (AA genome) and *Oryza brachyantha* (FF genome).

POSTER

Poster # 99

THE COMPLETE SEQUENCE OF THE CHLOROPLAST GENOME OF ORYZA BRACHYANTHA

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We sequenced the chloroplast genome of Oryza brachyantha from a BAC clone containing the entire organelle DNA. We screened high-density filters from the BAC library of the wild rice species with three chloroplast gene probes. Ninety-six positive clones were randomly selected, fingerprinted using the HICF methodology, assembled in contigs with FPC software (Arizona Computational Genomics Laboratory) and end sequenced. These analyses allowed selecting two clones that potentially contained the entire chloroplast DNA. Shotgun libraries from both clones were constructed, and one of them submitted to sequencing. Sequencing reads were assembled using CONSED software until a single sequencing contig was obtained. In silico digestions of the sequence match the patterns observed with four restriction enzymes. The sequence, containing 134611 base pairs, was reshuffled to match the same organelle sequences from *O. sativa* and *O. nivara*, deposited in GenBank. CrustalW alignments revealed that the chloroplast genome of *O. brachyantha* shares 96.8 % of homology to the referred species of the genus. This sequencing is currently under annotation.

POSTER

Poster # 100

IDENTIFICATION OF RICE PROTEIN KINASES, PROTEIN PHOSPHATASES, AND MEMBRANE TRANSPORTERS

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My group has been working on identify and making a preliminary functional classification of three major groups of proteins: the protein kinase, the protein phosphatases, and membrane transporters. Together these groups comprise over 10% of the total genome. Functional assignments are made by comparison to other known plant (Arabidopsis) and animal species, and by examiniation of motif patterns. Tree representations of these data simply comparison of rice and Arabidopsis functional groups.

POSTER

Poster # 101

CELL-TYPE SPECIFIC IN PLANTA EXPRESSION OF TRANSGENES PROVIDED BY THE GAL4 ENHANCER TRAPPING SYSTEM IN RICE *(ORYZA SATIVA L.)*

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As part of the Génoplante library of rice insertion lines, we have generated more than 13,000 enhancer trap (ET) Nipponbare plants harbouring the pC4956:ET15 T-DNA. In this T-DNA construct, the gene encoding the yeast transcriptional activator GAL4 serves as the trapping element while the gene encoding green fluorescent protein (GFP), controlled by multimerized upstream activating sequences (UAS) to which GAL4 binds, serves as the reporting element . Integration of the ET cassette into the rice genome often results in endogenous gene detection by cell-specific activation of gal4 and gfp by native genomic enhancers. In a first screening effort of the library, we observed the organs of T0 adult plants (1,982 lines), T1 seed (2,684 lines) and T1 seedlings (2,667 lines) for gfp expression. Nearly 30% of the lines produced GFP, and we identified lines with gfp expression in specific cells of all major organs of the rice plant. To demonstrate that UAS:geneX constructs can be transactivated in specific cell-types where gal4 and gfp are expressed in rice, we introduced a pC2300:UAS-GUS T-DNA in cells of seed embryo calli of 3 pC4956:ET15 lines exhibiting stable and contrasting patterns of GFP expression, through Agrobacterium co-cultivation. No GUS activity was detected in regenerating transgenic calli produced by Agrobacterium-mediated transformation of wild-type rice with this construct, demonstrating that, in the absence of activation by GAL4, the construct was incapable of driving gusA expression. Stable, GAL4-driven transactivation of gfp and gus was clearly demonstrated in the same specific cell-types of rice roots, leaves and flowers of regenerated T0 plants and their T1 progenies . GUS activity was only observed in GFP positive plants and seedlings and segregated in a mendelian fashion in T1 progenies. GFP and GUS patterns matched not only in terms of pattern type (root epidermal, floral trichomes, etc.) but also with respect to intensity,. GUS activity patterns were also very consistent among the several (20-80) transformation events derived from the same ET line. The results demonstrate that these enhancer trap lines present an ideal tool for precise, cell-specific expression of transgenes, or the manipulation of native gene expression using techniques such as RNAi silencing, in a model monocot.

POSTER

Poster # 102

DOES RAD50 PLAY A CRUCIAL ROLE IN THE DEVELOPMENT OF RICE?

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The rad50 gene of *Saccharomyces cerevisiae* plays a crucial role in meiotic recombination as well as DNA repair during vegetative growth. Rad50 interacts with the product of the Mre11 and Xrs2 genes to form a three-protein complex involved in the repair of DNA-double strand breaks (DSB). Yeast mutants lacking this complex are X-ray sensitive, defective in illegitimate recombination (IR) and show a weak hyper-rec phenotype for homologous recombination (HR) in dividing somatic cells. Recently, the Arabidopsis thaliana rad50 homologue gene has been cloned and intrachromosomal HR experiments involving homozygous and heterozygous mutants for this gene have confirmed the hyper-rec phenotype.

Based on the sequence of the *S. cerevisiae* and *A. thaliana* Rad50 proteins, we isolated the rice cv. Nipponbare rad50 homologue gene. The rad50 genomic CDS covers 17,215 bp and is localised in the centromeric region of chromosome 2, near the C106 marker. Southern analysis of DNA prepared from rice cv. Nipponbare young leaves also suggested the rad50 gene to be single copy. Furthermore, we cloned a 4,114 bp full length cDNA from a poly(A)+ RNA extract isolated from rice cv. Nipponbare calli. Sequence comparison between this cDNA and rad50 genomic CDS allowed us to identify 27 exons which is the same number of exons that were identified in the rad50 Arabidopsis gene. The translated protein show a high similarity to the other known plant Rad50 proteins and shares some conserved domains. The annotated rad50 region included in the OJ1288_D09 BAC clone was submitted to Genbank (accession number BK004165, only available after publication) as well as the 4,114 bp sequence deposited as the rice cv. Nipponbare rad50 cDNA (accession number AY277897).

Northern analysis of rad50 transcription level using a probe corresponding to the 20th exon of the rad50 cDNA, revealed high transcription levels in fast growing tissues such as calli and inflorescence while weaker expression was observed in leaves and mature roots.

We identified a T-DNA insertion at the 3'-end of the 8th exon of rad50, i.e. early enough in the sequence to prevent the formation of an active Rad50 protein, in the Génoplante rice insertion line library. Apparently, the mutant line can be maintained only in a heterozygous state, suggesting that Rad50 may be crucial in rice development. This tentative result agrees with that found in vertebrates but not in yeast and Arabidopsis.

POSTER

Poster # 103

GENERATION, CHARACTERIZATION AND EVALUATION OF THE GéNOPLANTE RICE INSERTION LINE LIBRARY

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We have completed the generation of an insertion line library through Agrobacterium-mediated transformation in the model cereal rice, cv. Nipponbare. The library encompasses 30,000, 3,000 and 13,000 lines harbouring a T-DNA equipped with a gusA enhancer trap (pC-4978), a gusA enhancer trap and a Ds element (pC-4984) and a gal4:gfp enhancer trap (pC-4956ET15) respectively. As each line contains an average of 2.2 copies of the T-DNA at 1.4 locus and of 3.2 new copies of the retrotransposon Tos17, the collection eventually totalizes more than 200,000 inserts, ensuring a good coverage of the rice genome and the possibility of creating additional inserts in specific chromosomal regions using Ds launching pads. Parallel systematic walk-PCR based amplification of regions flanking the left border of T-DNA inserts has been conducted. Over the 35,685 flanking sequences which have been generated, 19,786 (55.5%) represented good sequences larger than 30bp (average length 250 bp). 90.5 % of these good sequences were assigned to at least one position in the IRGSP Nipponbare genome sequence. T-DNA insertions appear to be randomly distributed over the 12 chromosome pseudomolecules, followed that of the FL cDNAs along the chromosomes with a lower insertion density around the centromere region and a higher density in the subtelomeric regions. T-DNA inserts were found to rarely integrate into repetitive sequences. 47% of T-DNA inserted within an interval extending from -250 bp upstream the ATG to the STOP codon of predicted genes of Chr.1, thereby generating reliable knock outs. Preferential insertion was also observed within the first 250 bp upstream the putative ATG start codon. Using 4 kb of sequences surrounding the insertion points, 62% of the sequences showed significant similarity to gene encoding known proteins (E-Value <1.00E-05). Along the same line, 40.4% of the T-DNA FSTs match the intervals framed by the limits of the 21,707 FL cDNA, used to establish a gene ontology-based functional classification of genes (Kikuchi et al 2003, Science 301, 376-379). Moreover, no bias is detected in any of the functional categories of classified FL cDNAs interrupted by a T-DNA insert. Based on these results, we estimate that at least 8,000 rice genes are tagged in our ca 20K T-DNA FST population. Current effort aims at increasing the size of the FST population by sequencing the right border of T-DNA inserts. Large scale screenings of the lines for presence of GUS/GFP, grain filling, response to Magnaporthe inoculation and morphological/ physiological changes are conducted under parallel projects. Mutant phenotypes, excluding pigmentation alterations and segregating in a Mendelian manner, are detected in 1-3% of the lines in the 3 independent screens, which already included more than 5,000 lines. Parallel seed increase and evaluation of the first 10,000 lines has been conducted under field conditions at CIAT, Colombia allowing the detection of ca.17% of clear-cut mutant phenotypes. Based on these evaluations, a tagging efficiency of the observed alterations by T-DNA and Tos17 inserts is being established through molecular analyses of progenies. To integrate the sequence and phenotype data generated by these projects, we have developed Oryza Tag Line, (http://genoplante-info.infobiogen.fr/oryzatagline/) consisting in a phenotype database focusing on phenotypic and reporter gene expression data and a related genomic database based on the FlagDB++ structure(Samson et al. 2004 Nucleic Acids Res. 32:347-350) which integrates the insertion line FST data in their annotated genome environment searchable by BLAST query.

POSTER

Poster # 104

ORYZA TAG LINE, A DATABASE FOR THE PHENOTYPIC CHARACTERIZATION OF THE GéNOPLANTE RICE INSERTION LINE LIBRARY

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To organize data resulting from the phenotypic characterization of the library of T-DNA insertion lines of rice (*Oryza sativa* L cv. Nipponbare) generated under the French genomics initiative Génoplante, we developed the Oryza Tag Line database. This database currently describes the phenotypes of 5,000 insertion lines which have already been evaluated under field conditions. As the T-DNAs used to generate the library were equipped with either a gusA or a gal4:gfp enhancer trap system, trapping of enhancer element may also result in detection of GUS activity or GFP fluorescence in assayed plant organs. Through a user-friendly interface, Oryza Tag Line currently displays agro-morphological and reporter gene expression records. Graphical web interfaces have been developed to facilitate forward genetic search either by keyword, trait and developmental stage, referenced mutant type, or site and level of reporter gene expression. In the long term, the database will contain information of whole library of insertion lines which have been produced in the frame of the project (Sallaud C. et al., 2003 Plant J 39:450-464). In parallel and to link phenotypic and sequence information, isolation and sequencing of T-DNA left border flanking regions (Flanking Sequence Tags or FSTs) is carried out systematically and displayed through another Génoplante database: the FLAGdb++ module ((Samson F. et al. 2004 Nucleic Acid Res., 32:347-350); which has been adapted to rice. 2,000 of the 5,000 lines currently available in Oryza Tag Line possess FST sequences. . Information related to 10,000 additional lines should be available in a year time frame. Oryza Tag Line is accessible for consultation via the internet web site of Génoplante-Info (Samson D. et al 2003 Nucleic Acid Res., 31:179-182) at http://genoplante-info.infobiogen.fr/OryzaTagLine/

POSTER

Poster # 105

RESTORATION OF FERTILITY TO CYTOPLASMIC MALE STERILE GENOTYPES BY A SUBCLASS OF PENTATRICOPEPTIDE MOTIF-CONTAINING PROTEINS

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Rice, maize, petunia, and many other plant species exhibit cytoplasmically-inherited male sterility (CMS) encoded by the mitochondrial genome. One or a few nuclear Restorer of fertility (Rf) genes, when present in a CMS background, confers male fertility. In those species such as maize and rice, in which there exist more than one CMS-encoding cytoplasm, particular Rf genes can restore fertility to lines containing one cytoplasm but not to the other, and vice versa. In all CMS cytoplasms that have been analyzed at the molecular level, the lack of pollen development has been found to result from the presence of an abnormal mitochondrial gene that produces a toxic protein. Rf genes have been observed to reduce the expression of the various abnormal mitochondrial proteins that disrupt pollen development in particular species. We used map-based cloning to identify the single dominant Rf gene from petunia, which is known to decrease the abundance of protein encoded by the aberrant mitochondrial pcf gene present in the CMS line's mitochondrial genome. The Rf locus contains two highly similar candidate Rf genes, Rf-PPR592 and Rf-PPR591, both of which carry pentatricopeptide repeat (PPR) motifs (Bentolila et al., 2002). The PPR motif is a 35-amino acid degenerate repeat that can be detected in over 400 predicted proteins in Arabidopsis and 600 in rice. Rf-PPR592 but not Rf-PPR591 restored fertility in transgenic petunia. Genes that restore Brassica/radish CMS lines and the Rice Boro-type CMS have subsequently been cloned by other groups and have been found to encode PPR motif proteins with significant similarity to the petunia Rf gene. The PPR motifs of the three known restorer genes contain consensus sequences that differ from the general consensus of PPR motifs present in many Arabidopsis and rice genes. In addition, homologs of the petunia restorer in rice and in Arabidopsis (a species lacking a Rf/CMS system) form the largest physical cluster of PPR-containing genes. Thus, restorer genes appear to belong to a specific subclass of the PPR family, a fact which should assist cloning of loci that restore fertility to additional CMS systems, such as the Rice WA-CMS cytoplasm, for which the identity of the restorer gene remains unknown. Research supported by the USDA NRI and the Rockefeller Foundation.

POSTER

Poster # 106

USING OVERGO TECHNOLOGY TO CREATE COMPARATIVE PHYSICAL MAPS IN THE GENUS ORYZA

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Oryza sativa L., commonly known as rice, is a main source of nutrition for over one-third of the world's population, making it arguably the most important agronomic plant species in the world. International collaborations in research have resulted in a complete sequence of the rice genome, an important first step toward improving the agronomic and nutritional quality of this plant throughout the world. Using the sequence data as a tool, we have designed overgo probes from chromosomes 1, 3, and 10 of rice and are using those probes to rebuild the rice chromosomes in 11 other *Oryza* species. This information will be an important tool in understanding the evolution, development, and genome organization of rice. Over 3,500 overgos have been designed spanning the three rice chromosomes, and 948 of these overgos have been used to screen BAC libraries of O. sativa and the other wild rice species. Using this data, as well as BAC end sequencing data from the wild species, we have been able to assemble positive BACs into contigs and align those BACs with the O. sativa reference sequence, giving us a picture of the differences in genome structure throughout the Oryzeae.

POSTER

Poster # 107

THE *ORYZA* BACTERIAL ARTIFICIAL CHROMOSOME (BAC) RESOURCE: A GENOME WIDE COMPARATIVE GENOMICS TOOL TO INVESTIGATE MONOCOT PLANT EVOLUTION, DEVELOPMENT AND PHYSIOLOGY

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We report the successful development of the first and most comprehensive set of bacterial artificial chromosome (BAC) libraries as a fundamental public resource from one cultivated and eleven well characterized wild species Viz *O.glaberrima*, *O.rufipogon*, *O.nivara*, *O.punctata*, *O.offcinalis*, *O.minuta*, *O.alta*, *O.austreliensis*, *O.brachyantha*, *O.granulata, O.ridleyii*, and *O.coarctata*, that belong to all the ten established genome types of the genus *Oryza,* to study monocot plant evolution, physiology and development. In brief, we have produced eleven 10X genome equivalent Oryza BAC libraries, comprising a little over one million BAC clones (1,004,544) that are arrayed in 2616, micro titer 384 well plates. We will present data on the detailed characterization of each *Oryza* BAC library, estimated genome sizes and organization of these genomes based on BAC-end sequence data. BAC libraries, filters and clones for this Oryza resource are available through the AGI BAC/EST Resource Center at www.genome.arizona.edu on a cost recovery basis.

POSTER

Poster # 108

THE ORYZA SATIVA NO POLLEN (OSNOP) GENE, ENCODES A C2-GRAM DOMAIN PROTEIN AND PLAYS A ROLE IN MALE GAMETOPHYTE DEVELOPMENT

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Phenotype screens of Ds insertional lines identified a male sterile Orysa sativa no pollen (Osnop) mutant with a pollen-less phenotype at the flowering stage. The mutant phenotype showed linkage to Ds insertion into Osnop gene. This mutant contained a deletion of 65 Kb chromosomal region at the site of Ds insertion containing 14 predicted genes. Out of these deleted genes, Delegen 5-7, 9-10 are functionally redundant with two or three copies with 100% homology in other regions of rice genome. The expression analysis showed that Delegen 8 did not express in normal growth condition, and Delegen 12 was expressed only in roots, thus deletion of these genes may not affect the pollen development. Our data and analysis also ruled out the possibility of delegen 1-4, 11, and 13 as candidates contributing to the pollen-less phenotype. Further investigation showed that the Osnop (delegen 14) was expressed only in late stage of pollen development with the highest expression at the stage of pollen release and germination by RT-PCR, Northern blotting, in situ hybridization, and promoter-GUS transgenic plants. Thus, the knockout of Osnop gene is the best candidate for the pollen-less phenotype in the mutant. Our data suggest that Osnop may play an important role during late stage of pollen development and its germination. Since the Osnop gene has both C2 and GRAM domains, it can be assumed that this gene cross-links both calcium and phosphoinositide signaling pathways. This is the first report to suggest possible functions for this gene in plant development.

POSTER

Poster # 109

PHYSICAL MAPPING AND SEQUENCE ANALYSIS OF RICE CHROMOSOME ENDS

Jianzhong Wu, Yuichi Katayose, Hiroshi Mizuno, Shoko Saji, Satoshi Katagiri, Hiroyuki Kanamori, Masaki Fujisawa, Nonukazu Namiki, Takashi Matsumoto and Takuji Sasaki

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Telomeres are the structures that form the termini of linear eukaryotic chromosomes. They are responsible for priming replication at the extreme ends of chromosomal DNA molecules and for stabilizing the chromosome ends by preventing chromosomes from fusing. Physical maps on the end regions of rice chromosomes 1, 2, 6, 7, 8 and 9 were constructed with PAC/BAC and fosmid clones. By chromosomal walking using overgo probes, 7 fosmid clones were mapped to the ends of chromosomes 1S, 2S, 2L, 6L, 7S, 7L and 8S. These clones contained telomere-specific repetitive sequences (TTTAGGG) with various lengths. Although no fosmid clones that contained telomere repeats currently have been screened and mapped to the remaining chromosomal ends, physical sizes of these telomere gaps have been successfully measured with the fiber-FISH methodology using PAC/BAC and telomere repeats as probes. All of these telomere gaps were demonstrated to have an estimated size less than 70 kb, except for the end of chromosome 9S in which a large fragment of rDNA gene clusters was associated. In this symposium, we will present our latest results obtained from the construction of physical map and sequence analysis of telomere regions. Discussion on the composition and structure of rice telomeres as well as telomeric regions will also be given.

POSTER

Poster # 110

EMPIRICAL ANALYSIS OF TRANSCRIPTIONAL ACTIVITY OF RICE CHROMOSOME 4 REVEALED TRANSCRIPTIONAL PATTERNS ASSOCIATED WITH CYTOLOGICAL FEATURES

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The complete sequence of the cultivated rice species Oryza sativa genome provides an unprecedented opportunity to study and improve our cereal crops. An essential step towards deciphering the biological information encoded in this nucleotide-level data is to obtain an accurate catalogue of genes and their respective expression patterns. Here, we report an empirically analysis of the transcriptional activities of rice chromosome 4 using a tilling path microarray based on subclone fragments. Representative organ samples of rice and in vitro cultured cells were examined to catalogue the transcribed regions of rice chromosome 4, and to reveal organ- and developmental stage-specific transcription patterns. We give experimental support for 80% computationally annotated TIGR genes. Novel transcriptions in 1,643 previously annotated intergenic regions were also detected. Comparing with cytologically defined chromosome features revealed active transcriptional activities in euchromatic regions in juvenile stages. One the other hand, active transcription of transposable elements was detected in mature stage samples. Combination of transcriptional data, cytological results, and sequence features showed that heterochromatic regions of rice are associated with high density of transposable element genes, active transcription of transposable element genes in mature stage, and high evolution speed.

POSTER

Poster # 111

DIFFERENTIAL EXPRESSION AND HORMONAL REGULATION OF ARCHAEBACTERIAL TOPOISOMERASE 6 SUBUNITS A AND B HOMOLOGS IN RICE (*ORYZA SATIVA L. SUBSP. INDICA*)

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DNA topoisomerases (EC 5.99.1.3) are ubiquitous enzymes which induce transient breaks in the DNA allowing DNA strands or double helices to pass through each other and religate the broken strand(s) relieving the topological constraints of chromosomal DNA generated during many fundamental biological processes. Topoisomerase 6 represents the only member of type IIB subclass found in archaea with A2B2 heterotetrameric organization which generates ATP-dependent double-strand breaks (DSBs) with two-nucleotide overhangs. We have isolated and sequenced the full-length cDNAs encoding for the archaebacterial topoisomerase 6 subunits A and B homologs from rice using RT-PCR and RACE. The three topoisomerase 6 subunit A homologs in rice were designated as OsiTOP6A1, A2, A3 and subunit B homolog as OsiTOP6B. The intron position and phasing was found to be highly conserved among rice and Arabidopsis TOP6 genes suggesting their evolution from common ancestors. The BLAST search done within TIGR database of rice genome and Southern analysis showed that all the rice TOP6 genes are represented as single copy in rice genome. All the three subunit A homologs harbour the CAP and Toprim domains conserved in archaebacterial topoisomerase 6 and SPO11 homologs in eukaryotes like yeast and Arabidopsis. OsiTOP6A1 is predominantly expressed in pre-pollinated flowers formed of meiotic tissue suggesting its role in meiosis, similar to SPO11 homologs in other eukaryotes. OsiTOP6A2 is expressed in all tissues but to a lower level as compared to the other TOP6 rice genes. The transcripts of OsiTOP6A3 and OsiTOP6B were found to be abundant in all the tissues examined. The OsiTOP6A3 and OsiTOP6B transcript levels declined slightly on brassinosteroid treatment in light-grown seedlings. However, ABA treatment stimulated steady-state transcript levels of OsiTOP6A3 and OsiTOP6B many folds, indicating their role in ABA signaling. In addition, yeast two-hybrid analysis demonstrated that full-length OsiTOP6B could interact only with OsiTOP6A2 and A3, suggesting that they may represent archaebacterial homolog of topoisomerase 6A and B subunits. The functional validation of all OsiTOP6A and B subunits is in progress in transgenics.

POSTER

Poster # 112

THE ORYZA MAP ALIGNMENT PROJECT: THE GOLDEN PATH TO UNLOCKING THE GENETIC POTENTIAL OF WILD RICE SPECIES

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The genus *Oryza* is composed of two cultivated (*O. sativa* and O. glaberrima) and twenty-one wild species (Khush 1997; Vaughan et al 2003). Cultivated rice is classified as an AA genome diploid and has six wild AA genome relatives. The remaining fifteen wild species are classified into nine other genome types that include both diploid and tetraploid species. The wild rice species offer a virtually untapped resource of agriculturally important genes that have the potential to solve many of the problems in rice production that we face today such as yield, drought tolerance, salt tolerance and disease and insect resistance.

To better understand the wild species of rice and take advantage of the finished IRGSP genome sequence, we have embarked on a comparative genomics program entitled the "*Oryza* Map Alignment Project" (OMAP). The specific objectives of OMAP are to: 1) construct deep-coverage large-insert BAC libraries from 11 wild and 1 cultivated Oryza species (*O. glaberrima*); 2) fingerprint and end-sequence the clones from all 12 BAC libraries; 3) construct physical maps for all 12 *Oryza* species and align them to the IRGSP genome sequence; and 4) perform a detailed reconstruction of rice chromosomes 1, 3 and 10 across all 12 Oryza species. The alignment of the *Oryza* genomes will provide a genome-level closed experimental system for the genus *Oryza* that can be used as a research platform to study evolution, development, genome organization, polyploidy, domestication, gene regulatory networks and crop improvement.

To meet the objectives of OMAP we required the construction of 12 high-quality BAC libraries and robust and cost effective protocols to fingerprint approximately 1,000,000 clones and generate 2,000,000 BAC end sequences by September 2005. We have accomplished both of these goals and our production and mapping groups are now in full swing. To date, we have already fingerprinted and end-sequenced 6 of the 12 rice libraries and Phase 1 physical maps have been generated for these species that can be viewed at www.OMAP.org. We are presently focusing our efforts on reconstructing 3 AA genome species, *O. glaberrima* and *O. rufipogon* and *O. nivara*, with the latter two thought to be the progenitors of modern cultivated rice – *O. sativa.*

In this poster we will present: 1) a summary of our progress on the development of the public OMAP resource; 2) an overview of our high-throughput fingerprinting and end-sequencing pipelines; and 3) emerging results on the AA genome diploids with a focus on the analysis of the heading date 1 orthologous locus in *O. nivara*.

POSTER

Poster # 113

PROGRESS OF THE ORYZA MAP ALIGNMENT PROJECT (OMAP)

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The *Oryza* Map Alignment Project (OMAP) will provide the first ever closed experimental system to understand the evolution, physiology and biochemistry of a single genus in plants or animals. We will (1) align FPC maps of twelve representative wild genomes of rice, including diploids and tetraploids, to the sequenced *O. sativa* AA diploid genome, (2) construct high-resolution physicalmaps of rice chromosomes 1, 3 and 10 using a combination of hybridization and in silico anchoring, (3) reconstruct chromosomes 1, 3 and 10 by using the SyBr (Synteny Browser), (4) Gramene CMAP viewer and GMOD Gbrowse to assemble BES and FP contigs, and (5) use FISH to develop a global view of the organization and evolution of repetitive sequences of chromosomes 1, 3 and 10 in the wild genomes. Such a system will empower the scientific community to address complex, whole-genome-scale, scientific questions such as: genome rearrangement, evolution, domestication, disease resistance gene clustering, and regulation.

Current progress on BAC clone library fingerprinting, BAC clone end sequencing, genome alignments, construction of high resolution physical maps of chromosomes 1, 3, 10, and preliminary analysis of repetitive sequences will be presented.

POSTER

Poster # 114

RICE DELETION MUTANTS WITH ENHANCED GROWTH UNDER DROUGHT STRESS

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Deletion mutants are a useful resource for confirming gene function. Through field screening of over 3500 mutants derived from the popular rice cultivar IR64, we identified 6 independent gain-of-function mutants with enhanced tolerance of water deficit. While the characteristic phenotype of the gain-of-function mutants, continued growth and maintenance of green leaf area under drought, has been confirmed through repeated field testing, the mechanistic basis remains unknown. The mutants are no better than IR64 if stress develops rapidly (in less than 1 week) during the reproductive stage. Tests of hormonal sensitivity just after germination suggest that certain mutants differ from the wild type in their responses to ABA, ethephon, GA, or IAA. Some of the mutants exhibit greater seed dormancy than the wild type. A backcross of mutant 545 to the wild type was evaluated under stress in the F3 generation, along with the intercross of mutant 545 with mutant 547. Scores of leaf drying were collected, along with data on plant height, anthesis date, and yield. The mean yields of both the backcross and intercross populations exceeded the average yield of IR64. Recombinants with exceptional performance were identified. These will be confirmed in subsequent evaluations

POSTER

Poster # 115

TRANSCRIPTOME ANALYSIS OF SALT TOLERANCE IN RICE USING SSH-MICROARRAYS

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We investigated the potential of Suppression Subtractive Hybridization (SSH) combined with microarray analysis to identify candidate genes involved in adaptation to a progressive salt stress in two indica cultivars of rice known to be susceptible and tolerant to salinity. An adaptive salt-stress –i.e. stepwise increase of 10mM NaCl concentration every 3 hours till 90mM - was applied at the 4-5 leaf stage on plantlets of the salt susceptible (IR31785) and tolerant (Nona Bokra) varieties. We previously generated more than 22,000 SSH cDNA clones from 31 subtracted libraries from salt/osmotic-stressed and unstressed tissues (leaf, root, sheaths) of several varieties. After insert amplification and purification, the 22K clones were spotted on glass slides using an Amersham automated slide processor (Lucidea spotter) and a set of four hybridizations of mRNA probes labelled through indirect incorporation method, was performed for each experiment. Three time points (3h, 4 days and 10 days), three organs (root, leaf and sheath) and two genotypes (Nona Bokra and IR31785) were used for RNA hybridizations on the 22k microarrays. Slides were immediately processed in both Cy3 and Cy5 channels with an Amersham generation III array scanner. Comparison of salt tolerant and salt susceptible behaviours demonstrated striking differences between the two varieties but also between organs for a given genotype. In IR31785, differentially regulated genes resulted mainly from a massive down-regulation corresponding to a strong decrease of basal metabolic processes and growth, which later conducted to death. In Nona Bokra, most of the changes were first observed in roots with also additional variations at 10 days in leaf and shoot samples indicating adaptation. Sequencing of a first set of 741 clones showing significant variation of expression allowed identification of 246 unique sequences. A first selection of 15 candidate genes differentially expressed between Nona Bokra and IR31785 unravelled two major processes which may be involved in avoidance of sodium influx in the salt tolerant variety. As early as 3 hours and 10mM Na+, several genes of the glutamate synthesis pathway were down regulated in the tolerant variety. This may limit the oxidative stress and/or Na+ influx through non-selective cation channels, known to be controlled by glutamate concentration. At 4 days and 90 mM Na+ , there is an upregulation of genes involved in cell wall modification such as those belonging to the lignin biosynthesis pathway. Genes such as metallothionein, dirigent-like protein, excreted peroxidases and thionins were overexpressed. Specific isoforms were also observed in shoots and leaves. These changes in cell wall composition may limit sodium influx through the apoplast. Physiological implications of these results will be discussed.

POSTER

Poster # 116

DETECTION OF SINGLE NUCLEOTIDE POLYMORPHISM AND ITS APPLICATION IN RICE

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Single nucleotide polymorphisms(SNP) are the most abundant variations in the genome. They can contribute directly to a phenotype or can be associated with a phenotype as a result of linkage disequilibrium. Most conventional trait markers and molecular markers, such as restriction fragment length polymorphism (RFLP) and cleaved amplified polymorphic sequence (CAPS) markers, are based on SNPs or insertions/deletions. Because of their abundance and codominance, the use of SNPs as a marker system has the potential for providing the highest map resolution. Typing of SNPs has progressed remarkably over the last several years, making genome wide linkage analysis and molecular breeding rapid and efficient.

Last year, BGI and IRGSP released the full genome sequences of Indica(93-11) and Japonica(Nipponbare) rice respectively. We have analysed two sequences comparing IRGSP's BAC with BGI's scafolds in silico. We have found 15,303,476 mismatch sites and designed 95,887 primers with 50kb of average distance from two sequences which cover the alignment range of 389Mb and. The average PCR product size was 390 bp. Based on these sequence analysis, we have carried out SNP detection at the region of Xa1 gene in 16 susceptible and resistant rice varieties and found numerous polymorphic site.

In this poster, we will discuss the efficiency of some SNP detection methods used in this study and will propose the way how we can analyse the genotypes of populations related with some resistance genes.

POSTER

Poster # 117

RELATING MOLECULAR VARIATION TO FUNCTION IN RICE GERMPLASM, MUTANTS AND BREEDING POPULATIONS

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Natural genetic variation present in germplasm is the key ingredient for trait improvement, yet the relationship between such natural variation and phenotypic performance is difficult to ascertain because of the diverse genetic background. Induced mutations in an identical genotypic background are advantageous for phenotypic assays, yet they lack the diversity of alleles. At IRRI we attempt to integrate the use of mutant stocks, breeding lines, and traditional germplasm maintained in the International Rice GeneBank Collection (IRGC) to assign functions to genetic loci and determine the breeding value for specific alleles.

For natural germplasm, we work with a subset of diverse Oryza accessions derived from the over 110,000 accessions held in the IRGC. Other non-IRGC varieties that have been extensively characterized or used in breeding programs supplement the IRGC-derived accessions. For *Oryza sativa*, this subset spans the range of variety groups and eco-cultural types for improved and traditional varieties, landraces, and breeding lines. For related species, our panel of 96 wild relatives from the IRGC includes all of the species and genome types from the closely related AA to the distant HHKK genomes (e.g. from *O. rufipogon* to *O. schlechteri*). For mutant collection, we use a collection of 40,000 M4 lines of the indica variety IR64 produced by chemical and irradiation mutagenesis. This population has provided useful allelic series to identify and confirm candidate genes in positional cloning experiments. Novel variants conferring gain and loss of function in response to biotic and abiotic stresses have been identified in the collection.

Based on results from expression and mapping studies, we select a set of candidate genes involved in stress response pathways to assay allelic variation in the germplasm and IR64 mutants. For each gene, we design locus-specific primers for the upstream regulatory and coding regions and apply TILLING to survey haplotypic and SNP variation across mutants, *O. sativa* germplasm as well as wild Oryza species. In parallel, we evaluate the phenotypes associated with these alleles by systematic phenotyping. As a case study, we are using putative beneficial alleles to track the progress of phenotypic gain in breeding populations for disease resistance. We envision a systematic approach of associating phenotypes with genotypes through a broad sampling of induced and natural variation can reveal the functional relationships among regulatory and effector genes, and provide the needed information for maximizing the use of allelic variation in breeding.

POSTER

Poster # 118

A TILING-PATH MICROARRAY-BASED TRANSCRIPTION ANALYSIS OF RICE CHROMOSOME 10 FOR IDENTIFYING THE TRANSCRIPTOME AND RELATING TRANSCRIPTION TO CHROMOSOMAL ARCHITECTURE

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Genome sequencing of the two major subspecies of domestic rice (Oryza sativa), japonica and indica, is essentially completed. The abundant unusual compositional and structural features make accurate annotation of the rice genomes a challenge. We report here a comparative tiling-path microarray analysis of japonica and indica chromosome 10. This analysis provided expression support to 2472 (81.9%) of the 3019 annotated non-redundant protein-coding gene models in japonica and 2428 (86.4%) of the 2809 indica models. Integration of microarray and comparative gene model mapping results identified 549 additional expression-supported gene models in the japonica chromosome, representing an 18% increase of the annotated coding capacity. Additionally, cDNA cloning and tiling microarray analysis validated transcription of a significant portion of gene models without previous experimental support. These results also suggest that the predicted gene structure of about half of the previous unsupported models need correction. Furthermore, tiling microarray analysis revealed a functional correlation of chromosome-wide transcriptional activity with the chromosomal architecture.

POSTER

Poster # 119

PRELIMINARY STUDY ON THE RICE GENOME METHYLATION PATTERN

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Recent advances in epigenetic study suggest that DNA methylation and histone modification play important role in chromatin organization, gene regulation and development. With the completion of rice genome sequencing and the emerging of tiling microarray technology, it is possible to detect the DNA and histone modification sites on the whole genome scale. In this study, we intend to reveal DNA methylation pattern in the rice genome using the rice chromosome 4 tiling microarray, which is derived from a plasmid library for random sequencing. We isolated methylated DNA from the sonicated rice genome by the affinity between methyl-CpG binding domain protein and methylated DNA, labeled with Cy5, and co-hybridized with Cy3-labeled total genomic DNA. By now we have examined the methylation pattern in plant tissues such as light shoot, dark shoot, root and suspension cells. The overall degree of methylation in different tissues is similar, while local variations are frequent among tissues. Heterochromatin region has relatively higher methylation level than euchromatin region. In-depth analysis of the data is still under way.

POSTER

Poster # 120

MICROARRAY ANALYSIS OF GENE EXPRESSION IN LOW-NITROGEN STRESSED RICE

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The objective of this study is to identify genes that are related to uptake or assimilation of nitrogen. Seedlings of rice cultivar Minghui 63 were cultured in full nutrient solution for 2 weeks, and transferred to a solution with the nitrogen concentration reduced to one sixth of the full nutrient solution, with seedlings that were transferred to the full nutrient solution as the control. The roots of the stressed and the control seedlings were harvested at 20 min, 1 hr and 2 hr after the stress treatment. mRNA samples extracted from the low-nitrogen stressed seedlings and the control were reverse-transcribed and labeled with Cy3 and Cy5 respectively. The fluorescence labeled probes were hybridized with the microarray of 11520 unique genes from a Minghui 63 normalized cDNA library and five subtractive cDNA libraries. Each hybridization was repeated four times. A “yellow experiment” was conducted to determine the thresholds for declaring significantly increased or decreased expression. The results indicated that many genes changed their expression patterns when the seedlings were stressed with low-nitrogen. In all, 36, 67 and 103 genes respectively were detected as showing enhanced expression in stressed materials in 20 min, 1 hr and 2 hr after stress, and 2, 500 and 0 genes showed reduced expression. Functional analysis of these nitrogen starvation response genes shows that: (1) the genes involved in photosynthesis and energy metabolism were down-regulated rapidly; (2) many of the genes involved in early responses to biotic and abiotic stresses were up-regulated while many other stress responsive genes were down-regulated; (3) some of the genes for ABC transporters were up-regulated and others down-regulated; (4) regulatory genes including transcription factors and ones involved in signal transduction were up-regulated; and (5) the genes known to be involved in N uptake and assimilation showed little response to the low N stress. In addition, 138 (27.5%) of the down-regulated genes and 56 (35.0%) of the up-regulated genes were classified as genes of unknown functions.

POSTER

Poster # 121

DEVELOPING HYBRID RICE SEEDS

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Rice is the most important crop for more than half of the world population, providing a variety of essential nutrients for human diet. Starch, synthesized in the amyloplasts, is the major component of rice grains. In this study we set out to characterize the expressed proteome of amyloplasts in the developing hybrid rice seeds.

We first developed a method to purify the amyloplasts and marker enzyme assays were used to examine the purity and intactness of the isolated amyloplasts. We then optimized the 2D gel electrophoresis conditions. The proteins extracted from the isolated amyloplasts were separated by 1D and 2D gel electrophoresis and examined both by PMF and MS/MS techniques. The mass spectrometry data were searched against the NCBI and SWISS-PROT protein databases and also the translated rice genome database. Using these approaches we have identified 142 proteins from the amyloplasts in the developing hybrid rice seeds (9311, PA64S and their hybrid) and carried out some initial analysis.

POSTER

Poster # 122

EFFECTS OF TEMPERATURE ON GENES REGULATING FLOWERING TIME IN RICE

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When a plant will flower (flowering time) is determined by the age of the plant, and by environmental cues/factors that include temperature, daylength (photoperiod), nutrients, and stress. In this study, we are testing if the genes regulating flowering time in rice change their expression levels due to a lowering of ambient temperature. This temperature manipulation results in observable changes in flowering time. Rice plants grown at 20oC showed a delay of almost two weeks in flowering compared to those grown at 28oC or 24oC. We have selected rice genes that have been predicted or shown to have a role in the genetic network controlling flowering time. Many of these genes have been shown to peak at certain times of the day. Using real-time RT-PCR, we examined the circadian expression of the selected genes at three temperature settings—28oC, 24oC and 20oC—under a short-day photoperiod. We report here our findings on two clock genes, OsLHY and OsPRR1, and one of the upstream genes in the photoperiodic pathway, OsGI. The circadian oscillation of OsGI has been previously reported (Shin et al., 2004). The two clock genes, OsLHY and OsPRR1, show different oscillation phases: OsLHY expression peaks at dawn while OsPRR1 peaks at dusk. Transcript levels of these genes relative to GAPDH indicate that OsPRR1 is highly expressed throughout the plant's development while OsLHY is only moderately expressed. Gene expression relative to temperature shows that OsLHY levels are higher at 24oC, but is lower at 20oC. OsPRR1 levels, on the other hand, are kept low at 24oC but are slightly higher at 20oC. OsPRR1 levels are also relatively higher at panicle initiation than at other developmental stages. Our results on the oscillation phase of OsGI is consistent with the previous report: OsGI peaks at dusk. At 20oC, OsGI levels are particularly high when panicles are being initiated.

We are currently analyzing expression of these and other genes in plants grown under a long-day photoperiod. A mathematical model on rice flowering time will be generated subsequently from these expression data.

POSTER

Poster # 123

MAPPING QTL'S AND CANDIDATE GENES ASSOCIATED WITH DURABLE RESISTANCE TO RICE BLAST.

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We characterized the genetic basis of high level durable resistance to *Magnaporthe grisea*, Sacc in the rice cultivar Oryzica Llanos 5 (OL5) in two populations of F7 and F6 recombinant inbred lines (RILs) from crosses between Fanny (susceptible) and OL5. Two linkage maps were constructed using 250 molecular markers: SSR, RFLP and RGAs for the first population (120 RIL's) and 178 SSR for the second population (231 RIL's). Thirty one loci were distributed on 11 rice chromosomes in the first population and twenty six loci distributed on 8 rice chromosomes in the second population were associated with the quantitative expression of one of two resistance traits (lesion type and disease leaf area) to eight isolates from 5 different lineages of *M. grisea*. QTLs with the largest effects for the first population were on chromosomes 6 (LOD 3.2 explaining 67.7% of the phenotypic variance) and 8 (LOD 10.0 explaining 63.8.0% of the phenotypic variance). The largest effects for the second population were on chromosome 8 (LOD 30.8 explaining 62.9% of the phenotypic variance) and 2 (LOD 8.2 explaining 65.4.0% of the phenotypic variance). Those QTL with largest effect were linked to resistance gene Pi2 and Pi9 on chromosome 6, and Pi11 on chromosome 8, all of which are known to confer high levels of resistance to several M. grisea isolates. Other QTLs (LOD 2.0 – 22.3) explained 2.4-45.4% of the phenotypic variance. We identified an isolate, FL440, which appeared able to overcome all the major genes in OL5. We are in the process of fine mapping some QTL's identified with this isolated in advances backcrosses lines to characterize potentially non-specific genes. Therefore, the durable, broad spectrum resistance in OL5 is associated with major genes inducing hypersensitive reactions and minor genes causing less distinctive phenotypic differences.

POSTER

Poster # 124

FUNCTIONAL GENOMICS BY SENSE-RNAI

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RNA interference (RNAi) is widely used to investigate plant gene function. There are currently two principal strategies to exploit RNAi for functional studies. One is based on stable integration into the genome of a transgene carrying an inverted repeat of the target sequence that can produce dsRNA transcripts, while the other is based on incorporation of the target sequence into an RNA virus that replicates via a dsRNA intermediate, a method known as virus-induced gene silencing. Both have their own advantages and limitations. This study exploits a third mode of RNAi induction. This "third way" is based on the phenomenon of sense cosuppression, whereby a highly expressed sense transgene can trigger RNAi. We refer to the sense cosuppression mode of RNA interference as Sense-RNAi, abbreviated as "S-RNAi". Previous studies show that three approaches - strong promoter (Que et al., 1997), direct repeat (Ma and Mitra, 2002) and addition of an inverse repeat of the nopaline synthase 3' UTR downstream of the target coding sequence (Brummell et al, 2003) - substantially increase the frequency of S-RNAi. To further evaluate and improve the silencing efficiency of S-RNAi, we designed 8 S-RNAi vectors by incorporating one, two or all three approaches into each vector. Testing of these vectors on 10 Arabidopsis genes is currently undergoing. To date, the most effective approach is the 3' UTR inverted repeat approach. The available data show that five of six genes tested with the 3' UTR-IR method are silenced at frequencies ranging from 11-90% of transgenotes. Our ultimate objective is to engineer a S-RNAi based approach for high throughput functional genomics and forward genetics.

POSTER

Poster # 125

CHARCTERIZATION OF A CORE COLLECTION OF RICE GERMPLASM AND ELITE BREEDING LINES IN THE US WITH GENETIC MARKERS ASSOCIATED WITH COOKING QUALITY

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Rice molecular markers have been developed in the granule bound starch synthase gene (Waxy) that controls grain amylose content and the soluble starch synthase IIa (Alk) gene that controls alkali spreading value (ASV). Both of these factors are considered the major determinants of rice cooking quality and texture. This set of markers is now being routinely used by US breeding programs to expedite the development of improved rice cultivars that meet rice cooking quality standards as determined by the US rice industry. For the first time, these markers have been used to characterize a core subset of rice germplasm that is maintained by the USDA-ARS National Small Grains Collection. There are over 17,000 accessions in the rice NSGC collection with about 10 percent of the selections being represented in the core subset. This core of some 1600 accessions, derived from over 100 countries, were grown in Stuttgart, AR during 2002 for phenotypic and genotypic evaluation. Two single nucleotide polymorphism (SNP) markers associated with amylose content, one SNP marker associated with starch pasting properties, an intragenic microsatellite (RM 190) in the Waxy gene, and two SNP markers in the Alk gene, associated with ASV, were scored. Fourteen microsatellite alleles for RM 190 were identified; eight of which were rare, each being found in less than 5% of the accessions. SNP genotypes were highly correlated with amylose content and ASV phenotypic results. A combination of SNPs in Exon 1 and Exon 6 of Waxy clearly differentiated low (4-19%), intermediate (20-22%), and high (>22%) amylose classes. Eight percent of the core accessions were found to be heterogeneous for RM 190. These results demonstrate that molecular marker evaluation provides a valuable method for characterizing world germplasm that is not skewed by environmental error, reveals sample heterogeneity that may be obscured in phenotypic evaluation, and clearly identifies novel alleles within a continuous spectrum of phenotypes.

POSTER

Poster # 126

ASSESSMENT OF THE NUMBER AND ROLES OF MICRORNA GENES IN PLANTS

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MicroRNAs (miRNAs) are a class of small noncoding RNAs that regulate the expression of other genes. Computational methods estimated that the number of miRNA genes is nearly 1% of the total gene number in the genomes of *C. elegans, Drosophila*, and human. The percentage remains unclear in plants. We present here a genome-wide computational approach to identify miRNA genes in Arabidopsis thaliana and rice. We estimate that the upper bound of miRNA genes constitutes about 0.6% of the genes in the genome. Our procedure was sensitive to capture 39 of 43 reference miRNA genes from Arabidopsis used for training and identified 60 novel miRNA genes. 47 of the novel genes are either paralogs of known miRNAs or newly identified miRNAs reported recently. The remaining 13 genes represent one known and four new miRNA families that were verified by our Northern blots. Nine homologous rice miRNA genes in three of the four new families were also identified. Predicted conserved targets of the novel miRNA genes suggested diverse roles other than transcription regulation, including disease resistance and regulation of cell division. We hypothesize that plant miRNAs may function in the nucleus based on three lines of evidence: potential base pairing between miRNAs and pre-mRNAs, nuclear processing of plant miRNAs, and potential nuclear localization of Argonaute proteins that function in the RNA-induced silencing complex. Further analysis revealed extensive complementary between two of the four newly identified miRNA families. These putative miRNA:miRNA duplexes, which are first described here in plants, suggest that plant miRNAs may also have the potential to regulate non-mRNA targets.

POSTER

Poster # 127

EVALUATION OF NUCLEASE GENES AND ACTIVITIES IN RICE THROUGH BIOINFORMATICS AND PROTEOMICS

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Nucleases are essential enzymes that participate in maintenance of the genetic material, in development, and in stress response. However, our knowledge of plant nucleases is limited and the annotation of nucleases in the two sequenced plant genomes is incomplete. We are interested in understanding the molecular mechanisms that control nuclease-mediated developmental processes and stress responses in rice. As a first step toward this end, we are applying two different approaches (bioinformatics and proteomics) to identify and characterize rice nucleases.

The completion of the rice genome sequence allowed the identification of many genes that have been annotated as nucleases based on homology to known genes from other species. However, annotation strategies are automated and several genes are missed during the process or are wrongly annotated. Thus, individual analysis of gene families is necessary to ensure accurate annotation. Moreover, for genes with no obvious or limited homology to known genes, different methods of identification are necessary. We are using Hidden Markov Model (HHM) predictions in combination with Neural Network algorithms to identify new members of nuclease families based on sequence and structure conservation. In addition to classification into protein families, subcellular localization can be obtained by analysis of sorting signals in the predicted protein. Clues about expression can be found by identification of promoter elements that could confer regulated expression in specific tissues or under specific developmental stages or stress conditions. As an example of our pilot analysis we will present data on three families of RNases: RNase T2, PR-10, and Argonaute (AGO). Eight genes are annotated as belonging to the RNase T2 family in rice. PHI-BLAST and PSI-BLAST analyses failed to identify additional genes. Our analysis indicates that only four genes are potential RNases, while the other four represent a new subfamily that has lost RNase activity. This may reflect the acquisition of novel gene functions through evolution. We also analyzed genes belonging to the PR-10 family. In this case only eight genes were annotated as members of the family. PHI-BLAST and PSI-BLAST identified 23 PR-10 genes, including the eight already annotated. RNases in this family have not been characterized enzymologically. Using HHM and computer learning methods we are analyzing patterns of sequence and structure conservation to predict residues involved in the active site of the enzyme. Finally, at least 20 genes belonging to the Argonaute family were identified. AGO proteins have a RNase H domain, and they are essential for the function of the RISC complex involved in posttranscriptional gene silencing (PTGS). Currently, it is accepted that PTGS occurs in the cytoplasm. However, analysis of predicted sequences suggest that some AGO proteins are imported to the nucleus. Our results, combined with our data showing that miRNAs can target pre-mRNAs (see poster by Ru et al.), indicate that PTGS can also be a nuclear process.

The current rice annotation contains 120 genes described as nucleases. Our analysis of only three families indicates that about 50 % of the genes were not annotated as nucleases or were annotated as nucleases without having conserved active site domains. Nuclease activities that are not yet accounted for by annotated genes have been identified in other plants. Our conservative estimates indicate that the number of nucleases present in the rice genome could reach at least 200 genes. Current proteomics approaches used to identify novel nuclease activities and to confirm nuclease activities for genes identified by computational approaches will also be discussed.

POSTER

Poster # 128

ECOTILLING STRESS TOLERANCE GENES IN CULTIVATED AND WILD RICE

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EcoTILLING diverse germplasm allows SNP and haplotype discovery at loci of interest (Comai *et al* 2004 *Plant J* **37**:778-786). At IRRI, we are using this technique to characterize natural variation in diverse cultivated and wild germplasm selected from the International Rice Genebank Collection at candidate genes for abiotic and biotic stress tolerance. Candidate genes putatively involved in stress tolerance were identified through converging evidence taking into account functional annotation, altered expression, co-localization with QTLs, and/or allelic shifts under selection. Target loci include DREB1, trehalose 6-phosphatase (TPP) for drought tolerance and oxalate oxidase and 14-3-3 protein for blast tolerance among others. We have designed locus-specific primers for upstream regulatory and coding sequence regions using the Nipponbare genomic sequence. Our initial studies have used either a panel of cultivated *Oryza sativa*with representatives from the indica, aus, deepwater types III and IV, aromatic, and japonica variety groups or a panel of wild *Oryza* accessions from all species and genome types. Reference lines for contrasts are Nipponbare (japonica type) and IR64 (indica type).

For the coding region of the TPP locus on CH2 at 109 cM (RGP map), 2 putative SNPs were detected in Kunmin Tsieh Hunan and Mudgo by either IR64 or Nipponbare when screening 96 *O. sativa* accessions. On the same accessions using upstream primers, the clearest mismatches were one apparently common to indica/japonica contrasts and three others in Kunmin Tsieh Hunan, Guta Bazail, and Loi Yai 62-40-140 by either IR64 or Nipponbare. TPP coding primers on 47 wild relatives resulted in amplicons for only AA genome species while upstream TPP primers also produced amplicons in CC, BBCC, and CCDD genomes. Contrasts of these to either IR64 or Nipponbare revealed a number of mismatches.

For the DREB1 locus at 16.1 cM, mismatches for indica/japonica contrasts were detected in both coding and upstream regions in sativa germplasm. Additional SNPs for the coding region were detected in Mimidam by IR64 and Taichung Native 1 by Nipponbare. For the upstream region, other mismatches were detected in Dagozan by Nipponbare and Dangar by IR64. On the wild germplasm for the coding region, amplicons were obtained across genomes as distant as GG but not in EE, HHJJ, or HHKK genomes. Many putative SNPs were detected including one present in japonica but absent from most wild and indica. While for the upstream primers, amplicons were only obtained for the AA genome and contrasts of these to IR64 or Nipponbare identified a range of mismatches.

A sampling of these and other putative SNPs are being sequenced to confirm the mismatches and establish the identity of the nucleotide difference(s). These results indicate the utility of EcoTILLING for identification of SNPs/haplotypes across both cultivated and wild Oryza. This report may well be the first example of the use of EcoTILLING to detect inter-specific differences.

POSTER

Poster # 129

A PCR-BASED SCREENING STRATEGY FOR DETECTING DELETIONS IN DEFENSE RESPONSE GENES IN RICE

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The challenge for the rice research community in the post-genome sequencing era is to identify the biological functions of the sequenced genes. Our focus is to use rice mutants to understand the functions of defense response (DR) genes in disease resistance. Rice mutant populations were induced by chemical (diepoxybutane) and radiation (fast neutron) mutagenesis in the indica variety IR64. To identify mutants in DR genes, a high-throughput PCR-based method using DNA pools of the mutant lines was developed. DNA from 8,350 deletion lines was organized into pools and primers corresponding to DR genes were designed and used to amplify the pooled DNA. PCR products were resolved in polyacrylamide gels and polymorphisms were detected by silver staining. We detected an individual mutant line with a deletion in a putative phenylalanine ammonia-lyase gene (PAL). The background of the mutant line was confirmed by microsatellite fingerprinting. Preliminary analysis indicates that the PAL deletion cosegregates with an intriguing phenotype characterized by a shortening and thickening of the internodes in tillers formed after flowering. More importantly, inoculation studies in the greenhouse indicate that PAL-deletion lines are more susceptible to Xanthomonas oryzae pv. oryzae than the wild type plants. Advanced generations of seed are being used to confirm the association of the PAL deletion with the intriguing phenotypes.

POSTER

Poster # 130

THE HISTORY OF THE R LOCUS REGION IN RICE, SORGHUM, AND MAIZE, AN EXAMPLE OF GENE MOBILITY.

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The r1 and b1 genes of maize, each derived from the chromosomes of two progenitors that hybridized more than 4.8 million years ago (mya), have been a rich source for studying transposition, recombination, genomic imprinting, and paramutation. To provide a phylogenetic context to the genetic studies, we sequenced orthologous regions from maize and sorghum (>600 kb) surrounding these genes and compared them with the rice genome. This comparison showed that the homoeologous regions underwent complete or partial gene deletions, selective retention of orthologous genes, and insertion of non-orthologous genes. Phylogenetic analyses of the r/b genes revealed that the ancestral gene was amplified independently in different grass lineages, that rice experienced an intragenomic gene movement and parallel duplication, that the maize r1 and b1 genes are descendants of two divergent progenitors, and that the two paralogous R genes of sorghum are almost as old as the sorghum lineage. Such sequence mobility also extends to linked genes. The cisZOG genes are characterized by gene amplification in an ancestral grass, parallel duplications and deletions in different grass lineages, and movement to a non-orthologous position in maize. In contrast to gene mobility, transpositions occurred mostly in the maize and rice regions recently (<3 mya).

POSTER

Poster # 131

TILLING FOR INDUCED SNPS IN RICE LIPASE GENES

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Rice bran is an extremely abundant by-product of the rice-milling industry. Rich in nutritious proteins, vitamins, and antioxidants, it is nevertheless an underutilized resource because of its tendency to spoil rapidly. Spoilage is caused, in part, by a bran lipase(s) that releases free fatty acids from storage triacylglycerols. If the activity of this bran lipase(s) could be reduced or eliminated, novel applications and markets for rice bran would be created. Anawah is identifying rice lipases that may be responsible for bran rancidity. Next we are employing an efficient, high throughput technology to identify induced, novel SNPs (Single Nucleotide Polymorphisms) in these lipase genes. Our method, called TILLING (Targeting Induced Local Lesions In Genomes), is a powerful reverse genetic screen that combines mutagenesis with targeted identification of induced alleles in specific genes. We will use this method to identify novel lipase mutants that lack or have reduced enzymatic activity. The availability of rice lines with reduced bran lipase activity will enhance the value of the bran and lead to the development of new uses and markets for this product.

POSTER

Poster # 132

GENERATION OF ACTIVATION TAGGING LINES OF RICE AND CHARACTERIZATION OF SEVERAL MUTANTS

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We have generated 12,000 activation-tagging lines of rice. In these lines, a T-DNA that contains tetramer of the CaMV 35S enhancer followed by its minimum promoter at its right border was introduced. Analyses of the T-DNA flanking region showed that T-DNAs were inserted preferentially in the genic region in rice genome and transcription of the neighboring region were upregulated. Several mutants including lesion mimic, stripe, dwarf, and short grain were obtained in the activation-tagging lines. A lesion mimic mutant, which we designated Lesion mimic 1 (Lmm1), was further characterized. Lmm1 was dominant and the phenotype linked to the T-DNA insertion. An ORF is located about 500 bp downstream of the inserted T-DNA and the deduced protein, designated OsAT1, shows sequence similarity to an acyltransferase whose expression is induced by hypersensitive reaction in tobacco. In addition, transcription of the genes for PR protein was upregulated, accumulation of phytoalexins, both momilactone A and sakuranetin, was increased, and resistance to blast disease was potentiated in Lmm1. Further, we combined OsAT1 genomic DNA downstream of the 35S promoter and re-introduced it into rice. The lesion mimic phenotype was detected in the produced transgenic lines, which clearly indicates that the overexpression of OsAT1 caused Lmm1 phenotypes.
Next we analyzed the Short grain 1 (Sg1) mutant. When the T-DNA insertion is heterozygous, the grain is small, and when it is homozygous, the grain is very small, indicating that the Sg1 phenotype is linked to the T-DNA insertion. The Sg1 plant also shows semi-dwarf phenotype. A full-length cDNA lies 1.4 kb downstream of the inserted T-DNA. The deduced protein shows no homology with any known proteins. These results strongly suggest that transcriptional activation of this novel gene causes the Sg1 phenotype.

This work was supported by a grant from the Ministry of Agriculture, Forestry and Fisheries of Japan (Rice Genome Project MP-1203).

POSTER

Poster # 133

RESOURCES FOR GENOME STUDY FROM THE ARIZONA GENOMICS INSTITUTE BAC/EST RESOURCE CENTER

Dave Kudrna, Chris Mueller, Mei-Zhong Luo, Jetty Ammiraju, Nick Sisneros, Jayson Talag, Kiran Rao, Elizabeth Ashley, Angelina Angelova, Hye-Ran Kim, Yeisoo Yu, Cari Soderlund, and Rod Wing

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The Arizona Genomics Institute BAC/EST Resource Center provides affordable access to BAC and cDNA libraries, high-density hybridization filters and clones for the world wide genomics community. The Center serves as a primary platform to launch our BAC library construction, physical mapping, genome and EST sequencing and positional cloning projects. Biological resources from these projects are available to the public from our on-line ordering system at http://genome.arizona.edu/orders/. We also accept clones from the scientific community to be archived and distributed world-wide as well as perform custom jobs.

The Center operates 2 Genetix Q-bots (colony picking, library replicating and rearrarying, filter gridding) and 44 -80 freezers (clone archiving) that provide high-throughput and barcode traceable clone handling capabilities. Recent BAC library projects include:

1) BAC library production and distribution for “Healthy People 2010” (NIH: PI Wing, Co-PIs Tomkins and Soderlund). Recently completed BAC libraries include: wallaby (Macropus eugenii, ME_KBa), zebra finch (*Teaniopygia guttata*), nurse shark (*Ginglymostoma cirratum*), rat (*Rattus norveigicus*), *Drosophila ananassae*, *Drosophila mojavensis*, *Drosophila virilis* , snail (*Biomphalaria glabrata*,) and echidna (*Tachyglossus aculeatus*).

2) The Oryza BAC library project: A fundamental public resource to study monocot plant evolution, development and physiology (NSF: PI Wing, PI Co-PIs Soderlund, Tomkins, Jackson & Luo). Recently completed BAC libraries include: *Oryza rufipogon (*Nivara, annual*), O. brachyantha, O. alta, O. officinalis, O. ridleyi, O. punctata, O. minuta, O. australiensis, O. coarctata, O. granulata, O. glaberrima, and O. rufipogon* (perennial).

3) The Green Plant BAC Library Project (NSF: PI Mandoli, Co-PIs Wing, Soderlund, Tomkins, DePamphilis & Banks). Recently completed: BAC libraries for *Volvox carteri* , Acorus gramineus, *Nuphar advena*, and *Selaginella moellendorfii* .

POSTER

Poster # 134

OSLTI: A SMALL FAMILY OF STRESS-RESPONSIVE GENES IN RICE ENCODES FOR MEMBRANE PROTEINS ASSOCIATED WITH ABIOTIC STRESS TOLERANCE

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Results of physiological analyses of the response of several rice genotypes to low, non-freezing temperature enabled them to be separated into cold tolerant and cold intolerant groups, with CT6748 and INIAP12, respectively, selected as representatives of the groups. OsLti6a and OsLti6b, isolated and cloned from a subtracted cDNA library made from cold-stressed CT6748, showed high similarities to cold-regulated RC12 of Arabidopsis and blti101 of barley. Northern blots and RT-PCR analyses revealed that the genes were not only up-regulated by low temperature, but also by exogenous ABA, water-deficit stress, and salinity stress. With the exception of salinity, each of the abiotic stresses induced more rapid induction of OsLti6 genes in the cold tolerant genotype than in the cold susceptible genotype. Analysis of the oxidative products of the two genotypes suggested that INIAP12 is more salt tolerant than CT6748. Time of expression analysis strongly supported our conjecture that the genes are under the control of CBF5, a novel transcription factor isolated from rice seedlings that binds to C-repeat/dehydration-responsive elements. OsLti6a and OsLti6b code for hydrophobic proteins of 6.0 kDa and 6.2 kDa, respectively, with membrane spanning domains. Localization of the proteins in the membranes of cold-stressed rice seedlings was confirmed by western blots of fractionated cell components. No interaction with other proteins was detected by the yeast two-hybrid assay. However, a yeast strain expressing the OsLti protein showed higher survival and growth rate when grown under cold stress (12oC), osmotic stress (1M mannitol) and salt stress (1M NaCl) compared to the same strain not expressing the protein. Concurrence of tolerance to cold, as measured by electrolyte leakage, and expression of OsLti implies a role for the proteins in membrane stability during abiotic stress. Research into the function of OsLti continues, including overexpression and silencing strategies in tobacco and rice.

POSTER

Poster # 135

SYBR: DETECTING AND DISPLAYING SYNTENY OF FPC MAPS

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SYBR (SYnteny BRowser) is an integrated pipeline for detecting and displaying synteny between two species. At least one of the species must have an FPC map, while the other can either have an FPC map, or be fully sequenced. The pipeline takes as input the FPC map(s) plus all relevant sequence, e.g. BAC-end sequence, marker sequence, or sequenced clones. BLASTs are performed between all sequence combinations in the two species (the user may specify exactly which BLASTs to perform, and at what E-value threshold), and the BLAST output data is collected and analyzed. Syntenic regions for particular FPC contigs or for blocks of multiple contigs are located, and several types of display are generated, including dot-plots, side-by-side alignments, and detailed alignments of contigs showing all clones, markers, and BLAST hits. Identification of multi-contig synteny blocks requires that the contigs be anchored and ordered, while single-contig synteny can be found with no anchoring information. The system is illustrated using rice and the recently completed agarose-based maize FPC map, the editing of which made substantial use of SYBR visualizations.

POSTER

Poster # 136

FUNCTIONAL GENOMICS OF RICE SUSCEPTIBILITY TO BACTERIAL PATHOGENS

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This research is focused on the identification and characterization of genes in rice (*Oryza sativa*) that have an effect on its tissue-specific susceptibility to two closely related bacterial pathogens: *Xanthomonas oryzae* pv. *oryzae* (Xoo) and *X. oryzae* pv. *oryzicola* (Xoc). Xoo is a vascular pathogen that invades the rice xylem and causes bacterial leaf blight. Xoc is a non-vascular pathogen that colonizes the apoplast of the rice leaf parenchyma and causes bacterial leaf streak. Our central hypothesis is that rice tissue-specific susceptibility to both diseases can be explained partially by the host traits and by its differential (and possibly tissue-specific) responses to the pathogens. To assess this hypothesis, global transcription profiling using spotted oligoarray hybridization is being conducted in our laboratory to identify rice genes uniquely up- or down-regulated in response to Xoo and Xoc . Samples derived from a collection of en masse synchronous dip-inoculated plants on which suspended virulent bacterial cells were efficiently and uniformly dispersed. By characterizing differentially expressed rice genes we hope to identify those that condition susceptibility to Xoo and Xoc as one step forward to developing new strategies to control bacterial diseases in crops. In addition, as a resource for functional studies, we have constructed Gateway cDNA libraries of rice undergoing infection by either Xoo or Xoc. This poster summarizes our preliminary discoveries and future perspectives.

POSTER

Poster # 137

RAPID IDENTIFICATION OF CANDIDATE MARKERS BY DISCRIMINANT ANALYSIS ASSOCIATED WITH AGRONOMIC TRAITS AMONG INBRED LINES OF RICE

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The objective of our research was to evaluate the potential of Discriminant Analysis (DA) to identify candidate markers associated with agronomic traits among inbred lines of rice. Traditional QTL mapping techniques are commonly used to identify loci or intervals linked to traits of interest, but power and precision are often lacking in small mapping populations. Studies with human populations employ linkage disequilibrium (LD) mapping strategies to detect SNP markers associated with candidate genes or simply-inherited phenotypic traits, but non-reproducibility and spurious associations dilute the impact of this methodology. Robust, non-parametric models offer a viable alternative to QTL and Linkage Disequilibrium strategies for mapping markers in plants. A total of 218 lines originating from the U.S. and Asia were planted in field plots near Alvin, Texas in 1996 and 1997 and agronomic data were collected for 12 traits. DNA profiles of each inbred line were produced using 60 SSR and 114 RFLP markers. Traditional genetic distance and model-based methods revealed population structure among the lines. Marker alleles associated with all traits were identified by DA at high levels of correct percent classification within subpopulations and across all lines. Markers pointed to the same and different regions on the rice genetic map when compared to previous QTL mapping experiments. Results from this study suggest that candidate markers associated with agronomic traits can be readily detected among inbred lines of rice using Discriminant Analysis combined with other methods described in this report.

POSTER

Poster # 138

FUNCTION OF RURM1 ALLELES ON THE ACTIVITY OF THE RICE TRANSPOSON MINIATURE-PING (MPING)

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We found a tourist-like MITE named mPing in a mutable slender glume mutant (line: IM294), which was induced from the rice variety Gimbozu. The mutability was caused by the precise excision of mPing from a mutant allele at the Rurm1 (Rice-ubiqutin related modifier-1) locus. In successive generations of IM294, normal glume plants (excision at meiosis) and chimeric plants (excision at mitosis) for glume shape almost always appear with high frequency (ca.1%). Compared to the original variety, IM294 shows high excision frequencies at other mPing inserted sites. To clarify the effects of the Rurm1 locus on the activity of mPing, we investigated transcription activities of Rurm1 alleles and two ORFs of Ping and Pong, both of which are putative autonomous elements of mPing. The transcription activities of Rurm1 alleles were positively correlated with those of the two ORFs of Ping both in IM294 and the original variety. In addition, transcription activities of ORFs of Ping were significantly increased in IM294 at young panicles, while those of Pong did not show a significant increase. This suggests that the wild type allele at the Rurm1 locus inhibits the transcription of Ping, and the mutant type allele lost the inhibiting ability. Ping is considered still active in Japanese rice varieties, because there are great intervarietal differences in its chromosomal location. The varieties with a higher number of Ping copies always harbor a higher number of mPing copies. This indicates that the translocation of Ping and mPing occurred simultaneously. Thus we concluded that Ping influences the translocation of mPing and that Rurm1 may inhibit the transposition of mPing through inhibiting the transcription of ORFs of Ping.

POSTER

Poster # 139

DIFFERENTIAL EXPRESSION OF RICE ROOT GENES UNDER HYPOXIC CONDITIONS

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Rice is a model species and the understanding of its genome structure and function can help to elucidate correlate mechanisms in other grass species.One particular trait that could be useful to othere grasses would be flooding tolerance which enables rice to be cultivated in soils with low drainage capacity. We have studied the response of different rice genotypes to soil hypoxic conditions and the differential expression of genes in tolerant/sensitive genotypes. Early results from cDNA AFLP analysis of differentially expressed genes reveal that flooding responses can be detected by this technique and can be studied at the mRNA fraction level.

POSTER

Poster # 140

EPIGENETIC SILENCING OF MAIZE MUDR / MU TRANSPOSONS

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Transposable elements are DNA segments that can move and multiply within a genome. The Mutator (MuDR/Mu) transposable elements of maize are extremely active, elevating mutation frequency up to 100-fold. All Mu elements have closely related ~215bp terminal inverted repeats (TIRs), but each class has unique, unrelated internal sequences. The autonomous MuDR element controls the transposition of the Mu family in maize. The element contains inward promoters in TIRs that result in convergent transcripts for the mudrA (transposase) and mudrB (helper protein) genes. Stochastically during development, unlinked MuDR elements can undergo coordinate epigenetic silencing measured as a decrease in MuDR transcripts, no transposition, and cytosine methylation of TIRs of all Mu elements. This epigenetic state is inherited over many generations. Silencing is considered to be a plant defense system against transposons invasions. Because MuDR silencing progresses during plant development and over one or a few generations, it provides a unique system to address the mechanisms and progression of transposon silencing. DNA methylation is a key epigenetic determinant and important in regulating gene expression and genome stability in mammals, plants, and some fungi. We show that the distribution of cytosine methylation during MuDR silencing; modification starts in both TIRs and internal regions of endogenous MuDR elements, and using transgenic corn, that imposition of a heritable silenced state requires both a TIR and coding region in cis. After the completion of silencing, methylation of the TIRs including near the transcription start sites is extensive and the internal regions are also heavily methylated at symmetric and some asymmetric cytosines. In contrast to the heavy cytosine methylation of the silenced MuDR, there is almost no methylation of the genes carrying a MuDR insertion. The boundary of the cytosine methylation state is very clear. In elements lacking a TIR, this boundary disappears and methylation also occurs in the neighbor gene. These results indicate during MuDR epigenetic silencing, cytosine methylation initiates at relatively specific parts both in the TIRs and the internal regions. By an unknown mechanism, TIRs act as a boundary to restrict epigenetic modification to the MuDR transposon.

POSTER

Poster # 141

MGOS: A DATABASE FOR MAGNOPORTHA GRISEA AND ORYZA SATIVA INTERACTIONS

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MGOS (*Magnoportha Grisea Oryza Sativa*) is a database to explore the interactions between rice and *Magnaportha grisea*, where magnaportha grisea in the pathogen that causes the rice blast disease in rice. Towards this end, we are gathering data from the biologists on this project, entering it into MGOS, and developing interfaces in which the users can easily interact with the data. The data being entered is ESTs, Long SAGE, microarray, mutant and genome sequence.
The highlights of the system are:

- PAVE: a system for viewing the EST contigs assembled from one library, or contigs assembled from all libraries together.
- SAGE: the Long SAGE Tags are extracted from different libraries and an interface is developed for querying and analyzing the data.
- Mutant: the mutant data is downloaded from a PhenoDB, which is a tracking databases created in Ralph Dean's lab at NCSU. MGOS site displays the results.
- Microarray: the array design and experiments are entered into MGOS and are available for viewing.
- Genome Browser: we are using the genome browser from www.gmod.org to display the rice and magnaportha grisea genome sequences along with the ESTs and SAGE alignments.

MGOS is at www.mgosdb.org.

POSTER

Poster # 142

GENOMIC PALEONTOLOGY AND THE HISTORY OF ASIAN RICE (*ORYZA SATIVA*).

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The origin of rice domestication has been the subject of debate over several decades. We have compared the transpositional history of 110 LTR retroelements in the genome of two rice varieties, Nipponbare (Japonica type) and 93-11 (Indica type), from which the genomic sequences have been released. Using a genomic paleontology approach, we estimate the date of divergence between these two genomes at 0.9-2.1 Mya, which is unambiguously older than the date of domestication of the crop (10000 years ago, during the late neolithic). In addition, we confirm this first in silico analysis with a survey of insertion polymorphisms in a wide range of traditional rice varieties from both Indica and Japonica types. This experimental data provides an additional evidence that Indica and Japonica rice arose from two independent domestication events in Asia. We will discuss our results from the perspective of our current model of genome evolution in Oryza genus.

POSTER

PRELIMINARY CHARACTERIZATION OF XB10 AND XB15 IN XA21-MEDIATED SIGNAL TRANSDUCTION

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Perception of extracellular signals by cell surface receptors is important to eukaryotic development and immunity. Many of these receptors possess intrinsic protein kinase activity in their cytoplasmic domains and regulate transcription of target genes through phosphorylation events. The rice Xa21 gene encodes an receptor kinase (RK) with leucine rich repeats (LRRs) in the extracellular domain and is a key recognition and signaling determinant in the innate immune response, a pathogen defense pathway widely conserved between plants and animals. Yeast two hybrid (Y2H) screens identified several XA21 binding proteins (Xbs). These include Xb10, encoding a putative transcriptional regulator ; and Xb15, encoding a PP2c phosphatase-like protein. Preliminary characterization of these genes suggest that they play a key role in the XA21-mediated defense response.

POSTER

Poster # 144

PROTEOME ANALYSES OF SALINITY INDUCIBLE TOTAL-PROTEINS AND PHOSPHO-PROTEINS IN RICE (ORYZA SATIVA)

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Drought and salinity are the most common abiotic stresses that result in severe crop loss. To investigate how cereal plants respond to salinity stress, we examined the dynamic profile of rice total proteome during NaCl stress using Fluorescence 2-D Difference Gel Electrophoresis (DIGE) approach. Proteins that are induced or decreased during salinity stress have been identified and quantified. Meanwhile, we also examined the dynamic change of the phosphoproteome during salinity stress. A group of salt inducible phosphoproteins have been identified using mass spectrometry. In addition, the phosphorylation sites of several salt inducible phosphoproteins have been mapped.

POSTER

Poster # 145

ARGEBIOS: INTEGRATED ANALYSIS OF T-DNA MUTANT COLLECTIONS FOR RESISTANCE TO BIOTIC STRESS IN RICE

O. Faivre-Rampant, J-B Morel, C. Gonzalez, V. Verdier, L. Albar, C. Brugidou, G. Droc, E. Guiderdoni and P. Piffanelli

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We created a network, called ARGEBIOS (ARGEBIOS Agropolis Reverse GEnetics for BIOtic Stress), of four laboratories involved in the area of resistance to biotic stress resistance in rice to collectively analyse compromised phenotypes of T-DNA tagged lines identified by reverse genetics approach. Candidate genes were identified from transcriptome and proteome data generated in the four laboratories and from available data in the literature.
Mutant T2 rice lines in candidate genes are analysed for compromised resistance to host and non-host rice pathogens and genotyped to verify the linkage between the identified phenotype and the T-DNA insertion. So far 150 mutant lines were analysed for compromised resistance to the three rice pathogens and non-host resistance to M. grisea and mutant phenotypes were identified in up to 20% of T-DNA screened lines.
C. Brugidou's team is studying the molecular interactions between rice and rice yellow mottle virus (RYMV) to elucidate the mechanisms controlling sensitivity, tolerance and the resistance of rice to the virus, a problem that has been little studied for any phytovirus. RYMV is a sobemovirus, one of the most damaging rice pathogens in Africa. Using a combined transcriptome, proteome and bioinformatic approach, C. Brugidou's team has identified deregulation of host genome expression at the beginning of viral infection process. They identified 550 genes highly deregulated in Indica and Japonica cultivars involved in all functional categories and are using the ARGEBIOS set-up to functionally validate their implication in the viral infection processes.

JB Morel's team is focusing on defining the signalling components involved in durable host resistance to M. grisea strains in both Japonica and Indica subspecies. The screen of T2 lines is based upon a compatible interaction enabling to reveal both EDS (enhanced disease susceptibility) and EDR (enhanced disease resistance) type of mutants.

P. Piffanelli's team interest lies on the mechanisms leading to cross-species non-host resistance to M. grisea strains in rice. A high-throughput inoculation protocol and phenotypic analysis for compromised non-host resistance of rice Nipponbare to M.grisea strains attacking other monocots (e.g. wheat, Digitaria, barley) was set-up at CIRAD to identify signalling components involved in type I and type II cross-species resistance mechanisms.

V. Verdier's team focuses on the molecular mechanisms leading to resistance to bacterial blight caused by Xanthomonas oryzae pv. oryzae (Xoo). Various Xoo strains were tested for their reaction on the wild type Nipponbare. Screening assays were performed on 30 day-old rice plants by leaf clip inoculation. Symptoms were scored by measuring lesion lengths. Most of the strains induced a susceptible reaction while strain PXO339 (Phil strains) induced a moderate resistant reaction (MR). It was selected PXO339 strain to screen the T-DNA mutants looking at EDS-type phenotypes. Mutants EDS phenotypes were identified in 13% of T-DNA lines.

We are now undergoing high-throughput genotyping of the identified lines following a protocol combining Southern blot and PCR analyses to confirm the linkage between T-DNA and observed phenotypes.
The ARGEBIOS integrated approach aims at identifying novel signalling and effector components involved in broad-spectrum resistance to biotic stress in rice and in specific pathways leading to resistance to bacterial, fungal and viral pathogens. The comparative study of mutant lines with fungal, bacterial and viral pathogens and for compromised cross-species (non-host) resistance will enable to pinpoint shared signalling pathways likely to be potential targets to engineer durable field resistance to rice pathogens.

POSTER

Poster # 146

IN DEPTH MOLECULAR CHARACTERIZATION OF T-DNA AND TOS17 INTEGRATION PATTERNS IN THE GéNOPLANTE RICE INSERTION COLLECTION

Delphine Mieulet, Martine Bès, Claire Rouvière, Gaétan Droc, Donaldo Meynard, Emmanuelle Bourgeois, Christophe Sallaud, Emmanuel Guiderdoni and Pietro Piffanelli

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In the framework of the Genoplante project, we have produced a library of rice (cv. Nipponbare) lines containing T-DNA and novel insertions of Tos 17 retrotransposon. Using a subset of 400 primary transformants harbouring the pC-4978 T-DNA construct, we attempted to establish a relationship between T-DNA organization, walk-PCR patterns of flanking regions and nature of the FSTs. Thorough Southern blot analyses revealed integration of an average of 2.2 T-DNA copies per plant, associated with an often-complex T-DNA organisation resulting from sequence rearrangements.
Moreover, we observed that backbone vector sequences are often integrated in transformants harbouring multiple T-DNA copies. A highly efficient digestion-ligation walk-PCR protocol to amplify genomic sequences flanking both Right and Left T-DNA borders was developed for five restriction enzymes. Our study revealed that the analysis by walk-PCR with three enzymes enabled to amplify at least one Flanking Sequence Tag (FST) from 90% of the primary transformants. Comparative analysis of FST sequences obtained from both Right and Left T-DNA borders revealed that in approximately 25% of cases novel FST sequences were obtained. In the same subset of 400 lines we estimated that on average insertion of 3.2 new copies of TOS17 had occurred per primary transformant. A novel highly efficient protocol of selective amplification of newly inserted copies of Tos17 retrotransposon was developed and enabled us to recover at least one sequence flanking a newly-transposed TOS17 copy in 70% the lines.Walk-PCR based protocols for both T-DNA and TOS17 enabled to obtain on average 70% of monoband and 30% of multiband products for every restriction enzyme we used. The development of a pipeline of bioanalysis of the sequences derived from multiband products, enabled us to maximize the efficiency of our walk-PCR based technology. The results of this pilot study will serve as a guideline to efficiently analyse the entire rice collection and help us to understand the mechanisms of multiple T-DNA copies. A highly efficient digestion-ligation walk-PCR protocol to amplify genomic sequences flanking both Right and Left T-DNA borders was developed for five restriction enzymes. Our study revealed that the analysis by walk-PCR with three enzymes enabled to amplify at least one Flanking Sequence Tag (FST) from 90% of the primary transformants. Comparative analysis of FST sequences obtained from both Right and Left T-DNA borders revealed that in approximately 25% of cases novel FST sequences were obtained. In the same subset of 400 lines we estimated that on average insertion of 3.2 new copies of TOS17 had occurred per primary transformant. A novel highly efficient protocol of selective amplification of newly inserted copies of Tos17 retrotransposon was developed and enabled us to recover at least one sequence flanking a newly-transposed TOS17 copy in 70% the lines.Walk-PCR based protocols for both T-DNA and TOS17 enabled to obtain on average 70% of monoband and 30% of multiband products for every restriction enzyme we used. The development of a pipeline of bioanalysis of the sequences derived from multiband products, enabled us to maximize the efficiency of our walk-PCR based technology. The results of this pilot study will serve as a guideline to efficiently analyse the entire rice collection and help us to understand the mechanisms of integration of both T-DNA and TOS17 in the rice genome.

POSTER

Poster # 147

ORYGENESDB AN OPEN SOURCE WEB-ACCESSIBLE RESOURCE FOR RICE REVERSE GENETICS

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OryGenesDB (http://orygenesdb.cirad.fr) is a web-based resource for rice molecular geneticists. The aim of this Oryza sativa database was first to display sequence information resulting from the activities of our group, such as the T-DNA and Ds flanking sequence tags (FST) produced in the framework of the genomics initiative Génoplante [1] and the EU consortium Cereal Gene Tags [2] respectively, and to link this information with related molecular data from several resources (cDNA, Gene, EST...) on the rice genome. Genome Browser [3], a Web-based application for displaying genomic annotations and other features, is the core of our database.

OryGenesDB now contains 28,134 mapped public FSTs (17,853 Tos17, 7,070 T-DNA, 3,211 Ds), 113,525 annotated genes (60755 from TIGR, 32307 from IRGSP), 20464 cDNA (KOME), and 64,925 EST clusters (OsGI from TIGR) and related resources like molecular markers. We further developed a set of tools around Genome Browser to retrieve as exhaustively as possible information related to queries with several starting points. FST search can be done by keywords, domain or Blast. When the FST search is performed by keywords, the output Excel file displays all the matches with details on the number of FSTs, their positions, a link to Genome Browser and external related links. When the FST search is performed by BLAST of query(ies) sequences (s) against the O.sativa genome. By running WU-BLAST against the DNA or protein database of O.sativa, users can retrieve a specific sequence and localize nearby insertion lines. The resulting pages display the gene and insertion information. Personal sequence annotations can also be uploaded and viewed in the context of the Rice genome. Specific advantages of OrygenesDB will be discussed in comparison with other available rice molecular resources.

[1] C. Sallaud et al., Plant J, 39:450-464, 2004.
[2] Van Enckevort et al., Plant Mol Biol, in press
[3] L. D. Stein et al., Genome Res, 12: 1599-1610, 2002.

POSTER

THE LEMONT/TEQING RIL RICE POPULATION PROVIDES UNIQUE OPPORTUNITY FOR CORRELATING MOLECULAR DATA WITH PHENOMICS

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[5] USDA-ARS Dale Bumpers National Rice Research Center, Stuttgart, AR 72160 USA (posthumous citation)

Genetic maps and populations are the basic tool with which nucleotide sequence and phenotypic traits are linked; they are important tools for functional genomics research. A permanent rice gene-mapping population now consisting of 280 F_{16} recombinant inbred lines (RILs) derived from a cross between 'Lemont' and 'TeQing' is being used for efficient mapping of molecular markers, major genes, and QTLs by at least 15 private and public research groups both within and outside the USA. Because of genetic similarities between rice and other cereal species, this LQ-RIL population supports investigation focussed on several crops in addition to rice. With literature now containing reports of more than 200 Lemont and TeQing QTLs affecting grain and plant traits, this population offers unique opportunity to efficiently evaluate relationships between genes, QTLs, phenotype, morphology, physiology, biochemistry, and environmental differences. Most of these QTLs were mapped relative to 203 RFLP markers. The map has been enhanced with the addition of more than 100 microsatellite loci.

Among the presently available rice gene-mapping populations, the LQ-RIL population is unique in that a) it was the first to be well adapted to USA and other semi-tropical growing conditions, b) it exhibits marker segregation ratios that are less genetically skewed (closer to Mendelian expectations) than other mapping populations, and c) it consists of a larger set of progeny lines than other populations, allowing for identification of genes with smaller effect and for more precise estimation of marker/gene locations. Lemont is a high-yielding tropical japonica variety from the USA; TeQing is a very high yielding indica from China. As a subspecific cross, this progeny population exhibits relatively high marker polymorphism (approximately 80% of RFLP and SSR markers tested), yet also exhibits sufficient reproductive fertility to support large-plot investigation. The LQ-RIL population, supervised by Dr. Pinson, was developed collaboratively by the USDA and Texas A&M University.

POSTER

FINE MAPPING OF THE WIDE COMPATIBILITY GENE S5N IN RICE

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Wide compatibility gene (WCG) is a very useful germplasm that enables the utilization of the strong heterosis between Indica and Japonica subspecies in rice. The overall objective of our study is to clone the WCG (previously identified as S5n), following a map-based cloning approach. Using an F1 population of a three way cross 02428/NJ11//Balilla, we previously located the S5 locus to an interval of 2.0 cM in length between markers R2349 and RM253 on chromosome 6. Using these two markers as the starting points, we constructed a contig map of the S5 genomic region by chromosomal walking, which consisted of six BAC clones spanning about 500 kb in length, and was subsequently reduced to 250 kb. For further reduction of the target region, we constructed an F1 population of 7000 individuals from the same three-way cross. Recombination analysis of highly fertile individuals from this population narrowed the region to 40 kb between two subclones, 7B1 and 15D2.

To reduce the noise caused by the background segregation, we developed two NILs of the S5 locus under the genetic background of Nanjing 11, with the S5 alleles derived from Balilla (line B) and 02428 (line 0), respectively. A three-way cross was made using these NILs (0/NJ11//B), from which an F1 population of more than 8000 individuals was obtained. Based on the results of a different study conducted in our group that S5 is a locus specifying embryo-sac fertility of indica-japonica hybrids, we investigated the individuals of the population based on embryo-sac fertility. Molecular marker mapping again located the S5 locus to the same 40 kb region between the two subclones 7B1 and 15D2. Functional analyses of the candidate genes by transformation and comparative sequencing are in progress.

POSTER

ANALYSIS OF TRANSGENE SILENCING, DEVELOPMENTAL TIMING OF TRANSPOSITION AND RECOMBINATION IN DS INSERTIONAL LINES

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Ac/Ds transposon mutagenesis is widely used for functional genomics in plants especially rice. However, silencing of the Ds elements in rice insertion lines has been reported by several labs questioning the applicability of such approach in later generations. In addition to this, transgene silencing, especially bar gene has been shown to occur in monocots including rice. We have performed a systematic analysis on various aspects of the silencing process. In order to examine silencing in the starter lines we have crossed 2 single copy Ac and five Ds T4 parental lines. High somatic transposition frequency (78 %) was observed in all of the ten cross-combinations by GUS assay. The average germinal transposition frequency of Ds found to be 41% by analyzing progenies of 68 F1 families for unlinked transposition events. Both of these frequencies are similar to those observed in earlier generations, respectively. We have used bar gene as a transposition marker and its silencing was analyzed using several thousand Ds insertion lines. Strikingly, bar silencing was very minimal in our system (<1 %) when Ds lines were propagated from F4 to F6 generations. Timing of transposition during plant development is an important factor, which determines the number of independent insertions among siblings of a F2 family. In order to determine the timing of transposition in rice, we have analyzed transposants from each tiller/panicle of an F1 plant and compared with each other. The transposants within the same panicle were identical and between the panicles were different, indicating that the transposition events occurred at post-tillering stage. The possibility that these independent events could be the results of a primary transposition followed by secondary events was ruled out by analyzing possible footprints with reciprocal PCR. Our results confirmed that the independent transposition events observed among panicles are due to primary transpositions. Analysis of Ds flanking sequences of independent transposants in a 14-kb long hot spot region revealed cluster of deletion which occurred at precisely the same position of the genomic DNA and the Ds truncated end in 9 out of 13 lines, indicating transposon mediated deletion via recombination.

POSTER

Poster # 151

ISOLATION AND FUNCTIONAL CHARACTERIZATION OF EXTRA-REDUCED FLAGLEAF (OSERFL) GENE, ENCODING A CYCLIN IA OF ORYZA SATIVA

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An Oserfl mutant was obtained in a phenotype screen of Ds insertion lines. The mutant displayed a small additional flag leaf when compared with wild type. The Ds flanking sequence was isolated and the BLAST analysis using the public databases revealed that the Ds was inserted into the exon region of a cyclin domain containing cyclin Ia gene. Further analysis showed that this gene belonged to the B-type cyclin family. The segregation analysis by the herbicide Basta and the BAR-PCR analysis confirmed that phenotype was linked with Ds insertion. Southern blot analysis confirmed that the Oserf1 mutant had a single copy of Ds element. Expression Analysis by Northern blot showed that the Oserf1 transcript was present in all tissues tested except in roots. Transgenic plants harboring the Oserf1 promoter-GFP showed expression in the calli and the young flowers. The Oserf1 mutant phenotype was rescued by complementation of wild type gene. In addition to this, when homozygous mutant crossed with Ac transposase lines revertants were obtained which showed the wild type characteristics. Our results clearly demonstrated that this gene might play a role in flag leaf development in rice. Further analysis of Oserf1 gene functions is in progress.

POSTER

IDENTIFICATION OF ALLELIC VARIATION AT PUTATIVE CANDIDATE GENE LOCI ASSOCIATED WITH DROUGHT RESPONSE IN RICE: FUNCTIONAL GENOMIC APPROACH

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Drought stress response is a complex trait governed by many genes dispersed throughout the rice genome. Genomic resources are therefore needed to decipher the role of this multitude of genes and their functions on a genome wide basis. To this end, large scale ESTs were generated from drought stressed seedlings of an upland indica rice (Oryza sativa cv Nagina 22), which was used to identify genes associated with drought stress response in rice. This EST resource was also utilized to dissect QTL regions and identify sequences that are associated with drought tolerance phenotype in mapping populations. We have added 31 RFLP (27 ESTs+4 genomic clones) markers, 54 SSRs and 3 EST-PCR based markers to the CT9993/IR62266 rice genetic map using IR68586 DHL population. This revised map was used to identify several QTLs for morphological, phenological and yield related traits under field drought stress conditions. A large number of genes and members of different gene families have been identified from the N22 EST resource and mapped on to rice chromosomes, of which ~ 750 are classified as putative candidate genes for drought tolerance. A transcript map with ~2500 unigene sequences has been constructed, which provides a direct route for drought tolerance gene discovery and understanding gene structure. An EST microarray consisting of 7400 elements representing rice cDNA clones from normalized drought stressed library and also pearl millet cDNAs generated from differential stress libraries were constructed and hybridized with indirectly labelled total RNA. RNA used for hybridizations were made from seedlings grown in different regimes of water stress in a rainout shelter. Expression analysis is underway. In parallel, identification of the allelic variations in coding and regulatory regions of the putative candidate genes, and the construction of HapMap for drought tolerance using elite Indian genotypes is underway. We have analyzed 4 functional families of candidate genes for intraspecies sequence variation using gene resources of N22, Nipponbare genome sequence and WGS sequence of GLA4. To address thie problem of sequencing errors contributing to apparent SNPs, we developed a Perl script, gtCluster v1.0, for assessing redundancy with in the sub cluster. Also an automated platform was developed for SNP detection, storage and analysis and optimized for rice sequence data embedding gtCluster program. A total of 175 SNPs have been identified with high probability using Polybayes in the pipeline for SNP detection. Details will be discussed.

This work is financially supported by the Rockefeller Foundation, USA, and partly by the DBT, Govt. of India

POSTER

Poster # 153

GENETIC STOCKS – ORYZA (GSOR) COLLECTION

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Genetic stocks are useful tools both in applied plant breeding, such as locating traits in relationship to other traits with an identified marker, for speeding up research cycles with early-flowering mutants, and in basic genetic studies for determining gene location and function. Genetic stocks collections help preserve materials that otherwise might be lost as researchers retire and/or grants terminate. Model genetic stocks collections in the US have been set up in tomatoes, maize, barley, and wheat. In August, 2003, the Genetic Stocks – Oryza (GSOR) was established at the USDA-ARS Dale Bumpers National Rice Research Center, at Stuttgart, Arkansas.

The GSOR includes materials produced at Stuttgart such as dominant and recessive male sterile mutants and various morphological mutants. A japonica/indica cross mapping population (355 lines) was added in April 2004. Materials produced by cooperators, including other USDA-ARS units, universities, industry, NSF and NIR-supported projects, will also become part of the GSOR. The GSOR collection will make seeds and information for each stock available through GRIN to US and international rice researchers. The respository homepage (url = http://www.dbnrrc.ars.usda.gov/gsor/) will be maintained and will include information about the collection contents.

Donations to the GSOR are invited. Each submission should be accompanied by the following documentation: 1) Origin and/or pedigree; 2) Certification that seeds are disease free; 3) Material is provided with no intellectual property strings attached; 4) Permission from original donor for redistribution; and 5) Foreign-origin material must have come through APHIS-approved quarantine. Contributed seeds will be stored and distributed as long as stocks last, but not regenerated. All material will be subject to periodic review by the GSOR Liaison Committee.

POSTER

Poster # 154

COMPARATIVE STRUCTURAL GENOMICS OF THE PHOTOPERIOD SENSITIVE QUANTITATIVE TRAIT LOCUS HD1 IN GENUS ORYZA.

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In the last five years rice has emerged as an ideal model for comparative structural genomics in the grass family Poaceae. This has become possible because of a completely sequenced genome, a vast expressed sequence tag (EST) dataset, numerous annotation programs to predict structural characteristics of the rice genome and comparative mapping studies that reveal gene content and order conservation. The present study aims to understand the conservation of genic elements in a 120kb region encompassing the photoperiod sensitive heading date associated gene Hd1 in 12 species of genus Oryza, maize and sorghum. As a first step towards this goal, Bacterial Artificial Chromosome (BAC) libraries with greater than 10x coverage were hybridized with probes designed from the Oryza sativa cv. Nipponbare Hd1 gene and flanking EST sequences at a distance of 50-60kb from Hd1. BACs hybridizing to all three probes were chosen and are currently being sequenced. These sequences will then be run through gene prediction programs and compared to the 120kb region around Hd1 from *O. sativa* to determine gene order and content conservation. Secondly, BAC end sequences are available for *O.rufipogon, O.nivara, O.punctata,* and *O.brachyantha*. These are being mapped to the 120kb Hd1 region in order to identify BAC contigs with minimum overlap that span this region. Based on the sequence comparison data we plan to elucidate mechanisms of structural evolution in and around genes important to plant evolution and domestication.

POSTER

Poster # 155

SINGLE NUCLEOTIDE POLYMORPHISMS IN GENES AMONG JAPANESE LEADING CULTIVARS AND THEIR PARENTAL CULTIVARS OF JAPONICA PADDY-RICE IDENTIFIED BY PCR-RF-SSCP

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Some of SNPs (single nucleotide polymorphism) in genes is responsible for the phenotypic variations in plants. SNPs are also useful as DNA markers for the mapping of genes using a segregating population derived from a cross between closely related lines. Since pedigrees of many rice cultivars are known, SNP analysis of leading cultivars and their parental cultivars will enable the identification of some important alleles that have been selected in the breeding process. In the present study, DNA polymorphism of randomly selected genes in rice cultivars was analyzed by PCR-RF-SSCP (polymerase chain reaction-restriction fragment-single strand conformation polymorphism) technique. Out of the 1,500 primer pairs, 926 of them amplified single DNA fragments from genomic DNA of 'Nipponbare', 145 of which showed polymorphism between 17 Japanese rice cultivars. Among these cultivars, two, three, four, and five alleles were observed in 128, 8, 1, and 1 genes, respectively. The progeny cultivars inherited alleles exactly from their parental cultivars with a few exceptions. Combining the data on important traits of these cultivars and accumulated information on the genes in the chromosomal segments derived from each parent will enable the screening of the genes controlling some important traits.

Reference: Shirasawa et al. (2004) DNA Research 11: 275-283

POSTER

Poster # 156

RICE GENOME ASSEMBLY BY THE NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION

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Those BAC sequences which the International Rice Genomes Sequence Project (IRGSP) annotated as “Finished” have been assembled into the first build of the rice genome. 3290 sequence records of the 3498 records comprising the IRGSP minimal tiling path have been assembled into 195 contigs of 380 Mbp over 12 chromosomes. The assembly was performed so that the tiling path did not disrupt any gene features in the IRGSP annotation of the BAC. This allowed the assembled material to be annotated by transfering the features noted in the component BAC to the assembled record. This resulted in 48849 gene features with 37473 CDS regions being observed. Comparision between the chromosome 1 assembly done by IRGSP and chromosome 1 assembly done by NCBI shows the only differences are the position of the change from one BAC sequence to the overlapping BAC sequence in those instances where the NCBI process avoided disrupting an annotation feature in the component BAC record. The current rice genome RefSeq material is divided between chromosome 10, which was produced by TIGR, and the other 11 chromosomes, which were produced by NCBI.

The NCBI annotation pipeline has used the 203 thousand public rice EST records and the 54 thousand public rice full-length cDNA records to perform an independent feature annotation on the NCBI-assembled sequence. This endeavor will provide two opportunities. First, this will enable the comparison between the consequences of the current NCBI annotation algorithm and the IRGSP annotation algorithm. Second, this is expected to enhance the capability for plant-specific ab initio gene prediction at NCBI.

POSTER

Poster # 157

DISTINCT GLOBAL REORGANIZATION OF THE GENOME TRANSCRIPTION ASSOCIATES WITH ORGANOGENESIS OF EMBRYO, SHOOTS AND ROOTS IN RICE

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Plant cells have the capacity to differentiate into all the cell and organ types that constitute the adult plant. However, the molecular events during the process of differentiation are poorly understood, especially in monocot plants. We used a cell culture system to generate embryo colonies, then further induction into shoots and roots in Oryza L. subsp. indica (var 9311). The global transcriptional reorganization during the development of embryos, shoots and roots from cultured cells were studied by rice whole genome microarray. Overall, 1580 differentially regulated genes during embryo induction were identified, which contains genes working 90 metabolic pathways. During shoot and root organogenesis from embryos derived from culture cells, 1338 and 583 differentially expressed genes were identified that involve in 89 and 57 metabolic pathways, respectively. Comparison of these differentially expressed genes revealed little overlap during these 3 organogenesis processes. Analysis of over 20 representative genes identified from the microarray study by RNA blotting confirmed their microarray revealed expression patterns. These results indicate that distinct organogenesis involves specific reorganization of genome expression.

POSTER

Poster # 158

REGULATION OF RICE GENE EXPRESSION BY XANTHOMONAS ORYZAE PV. ORYZAE.

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Xanthomonas oryzae pv. oryzae (Xoo) is a causal agent of Bacterial Blight of rice. Our previous study has shown that the bacteria secreted multiple AvrBs3/PthA proteins into plant cells through Type III protein secretion system, and these proteins have a major role in bacterial virulence. The avrBs3/pthA gene family members encode three nuclear localization signals and a transcription activation domain, and both features are necessary for virulent function of the protein. Therefore, we speculated that the gene products activated host gene transcription and modify the host plant cell in the process of causing disease. We examined differential rice gene expression upon bacterial challenge using microarray, and found several rice genes are induced by Xoo and the gene induction pattern was disease specific. One of the induced genes, which encodes the general transcription factor II A gamma subunit (TFIIAg), was induced specifically by strain PXO99A. Two strains with mutations in members of the avrBs3/pthA genes of PXO99A showed loss of ability to induce TFIIAg and a reduction in virulence.

POSTER

Poster # 159

APPLICABILITY OF THE TARGETING STRATEGY TO MODIFICATION OF RICE GENES

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The gene targeting refers to the alteration of a specific DNA sequence in an endogenous gene at its original locus in the genome. We have succeeded in a precise and reproducible gene targeting by homologous recombination in rice (Terada *et al*., 2002 *Nature Biothech*.20;1030). Our basic strategy is the 'fishing out' of a targeting event rarely occurred.

A practical strong positive-negative selection has been carried out with a large-scale A*grobacterium*-mediated transformation using rice calli. In the first challenge of our gene targeting, the *Waxy* gene, which resides on chromosome 6 as a single locus, was selected. All the evidence confirmed the occurrence of the true gene targeting of *Waxy*. To access the applicability of our system, we have chosen the *Alcohol dehydrogenase* (*Adh*) gene *1* and *2* for the targeting modification because their genomic characteristics are different from those of the *Waxy* gene.

Adh1 and *Adh2* are located on chromosome 11 in the same orientation separated by 30 kb. The exon sequences of *Adh1* and *Adh2* are very similar. Between these *Adh* genes are *gypsy*- and *copia*-like retrotransposons and a hypothetical gene. We have applied our targeting strategy to the *Adh1* and *Adh2* genes separately. The several lines examined showed the *Adh2* gene was successfully targeted, and the frequency of the targeting of *Adh2* is comparable to that of the published *Waxy* gene. For *Adh1,* we are under characterizations. The applicability of our targeting strategy will also be discussed.

The work was supported by grants from The Ministry of Education, Culture, Sports, Science and Technology of Japan, and from The Ministry of Agriculture Forestry and Fisheries of Japan.

POSTER

Poster # 160

BREEDING AND GENETIC ANALYSIS OF A FERTILITY RESTORER LINE FOR A MALE-STERILE CYTOPLASM [CMS-CW] OF RICE.

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A male-sterile cytoplasm of rice has been first reported in a wild rice, Oryza rufipogon Griff. strain W1, by Katsuo and Mizushima (1958). The cytoplasm has been named [cms-CW]. The pollen grains of the male-sterile plants are well stained with I2-KI solution, but do not germinate on the normal stigma. Due to the lack of restorer genes among cultivars so far tested, this male-sterile cytoplasm has not been characterized further. Breeding of a restorer line (W1-R) was achieved by transferring the restorer gene(s) of W1 to a cultivar through anther culture. We are carrying out mapping of the restorer gene. The 1:1 segregation of fertile and sterile plants in BC1 population from a cross of W1-R and Taichung 65 demonstrates that the fertility restoration is controlled by a single dominant gene. The fertile seed set of all the F2 plants indicates that the fertility restoration functions gametophytically. Using CAPS and micro-satellite markers in the 96 BCF1 plants, the restorer gene is shown to be located on chromosome 4, on which no restorer genes have been reported. A fine mapping of the restorer gene and analysis of the mitochondrial genes are now in progress.

POSTER

Poster # 161

NATURAL VARIATION FOR MICRONUTRIENTS AND ANTI-NUTRITIONAL FACTORS: ARABIDOPSIS AS A MODEL

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Micronutrients in food (minerals, vitamins) are essential for human health. The quality of food not only depends on the absolute levels of these micronutrients, but also on the levels and types of anti-nutritional factors, which may affect bio-availability of micronutrients. Well-known examples of antinutrients are phytate (myo-inositol 1,2,3,4,5,6 hexakisphosphate) and polyphenols, inhibiting the intestinal uptake of e.g. Fe and Zn, due to complex formation. In regions where the diet is mainly based on staple crops (rice, maize, sorghum) people are at risk of mineral deficiencies. It has been estimated that over two-thirds of the world population is iron-deficient, resulting in anaemia in more than 30% of the population, largely in developing countries. Less clear figures are available for zinc-deficiencies, but recent publications indicate that the problem is equally relevant. In plant breeding programs, until very recently, hardly any attention has been given to micronutrient levels. Screening of available germplasm indicates ample natural variation for this trait in most crops, e.g., 3-fold for Fe content and 4-fold for Zn content in rice grains.

We have chosen Arabidopsis thaliana as a model species to investigate natural variation for micronutrient content and anti-nutritional factors, and the underlying genetic and physiological causes. Screening a series of Arabidopsis accessions revealed natural variation for most cationic minerals (Fe, Zn, Mg, Mn, Ca, K) and for phytate. Using recombinant inbred line populations, we were able to map quantitative trait loci (QTL) for all traits. Some of the loci for cationic minerals coincide, suggesting a common genetic cause of variation. Loci affecting phytate levels in seeds did not co-locate with loci for Fe or Zn, indicating that it is possible to breed for increased levels of minerals without raising the content of phytate. Comparing the locations of QTL with map positions of gene known, or suggested to be involved in cation transport, revealed that some QTL might be due to allelic variation in transporters and related genes, whereas other QTL might be caused by other, as yet unknown, genes. Fine-mapping of some loci is in progress.

POSTER

Poster # 162

APPLICATION OF PROTEOMICS IN GENE FUNCTIONAL STUDY USING RICE MUTANT

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Mutants are good materials for studying functional genomics especially when the whole rice genome has been sequenced. A novel mutation pool containing more than 3,000 mutants with wide variations on the same genetic background of the TNG67 rice variety has been developed by sodium azide mutagenesis at the Department of Agronomy, Taiwan Agricultural Research Institute. In the present report, we demonstrate the integration of two-dimensional (2-D) electrophoresis, mass spectrometry and bioinformatics to identify and characterize the function of protein in the early senescence mutant, SA0401. In our system, more than 1000 protein spots can be identified on a pI 4-7 2-D gel and several mutant specific proteins have been identified by LC MS/MS (ESI-Q-TOF). Several proteins were identified and found to be mutant-specific and corresponding to their characteristics. About 50 major leaf proteins commonly present in all leaf materials are found to involve in photosynthetic pathway providing the protein markers for construction of a basic protein picture for functional study of mutants. A senescence associate protein, OSSAP1, and its corresponding gene, a germin6-like gene, are specifically expressed in the recessive early senescence mutant, SA0401. By using the mutant and taking the advantages of rice genomic resources we are be able to study the relationship among gene, protein, function, and phenotype, this mutant-proteomic approach provides a new vision for the study of rice functional genomics.

POSTER

Poster # 163

ANNOTATION OF RICE GENE STRUCTURE AND ALTERNATIVE SPLICING USING AN ENHANCED PASA PIPELINE

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PASA, a Program to Assemble Spliced Alignments, was initially applied to improving the Arabidopsis genome annotation using maximal transcript alignment assemblies (Nucleic Acids Res. 2003 31:5654-66). Since its first release, the PASA pipeline has been extended to include the use of polyadenylation sequences to ascertain the orientation of transcripts lacking introns, as well as the automated annotation of poly-A sites in transcribed genes. Additional enhancements include gene splitting, gene merging, antisense transcript classification and routines for automated classification of alternatively spliced isoforms. The enhanced pipeline was used to update the gene models in TIGR Rice Genome Annotation project. Out of the 78,105 FGENESH based rice models, 19,722 models were updated. In addition, 3,720 alternatively spliced isoforms and 355 novel genes were created. PASA is a valuable tool in incorporating full length cDNA and EST evidence into genome annotation when genome-wide manual curation and updating is not feasible.

POSTER

Poster # 164

OSDR3 IS INVOLVED IN THE DEFENSE RESPONSES AGAINST BOTH BACTERIAL BLIGHT AND BLAST DISEASES OF RICE

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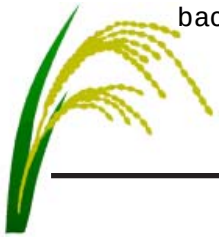
We identified a defense-responsive gene, OsDR3, from an indica rice cultivar, Minghui 63. The expression of OsDR3 was induced by both Xanthomonas oryzae pv.oryzae (Xoo) and Magnaporthe grisea causing bacterial blight and blast diseases, respectively. OsDR3 was predicted to encode a protein of 316 amino acids that had a conserved sequence of WRKY-type proteins, WRKYGQK, and a typical zinc-finger motif with C-X5-C-X23-H-X-H structure. In addition, OsDR3 protein contained a nuclear import signal. This gene was mapped to the long arm of chromosome 1 and its location corresponded to a QTL for disease resistance. To further characterize the function of the gene, we overexpressed OsDR3 in susceptible rice cultivar Mudanjiang 8 under the control of a maize ubiquitin promoter and suppressed the expression of OsDR3 in Minghui 63 using both antisense RNA and RNAi strategies. Totally 47 plants transformed with the overexpression construct were obtained. Thirteen of the 47 transgenic plants showed significantly ($P < 0.05$) enhanced resistance to the Xoo (PXO61) with the lesion area (lesion length/leaf length) ranging from 24% to 44% as compared to 62% measured for the control of untransformed Mudanjiang 8. The transgenic plants with enhanced resistance to Xoo also showed enhanced resistance to M. grisea. Northern blot analysis indicated that the enhanced resistance of the transgenic plants was correlated with the increased expressing level of OsDR3. Analysis of the promoter of OsDR3 connected with Gus gene revealed that the GUS activity could be induced after 6 h of inoculation of Xoo or after 4 h of treatment with salicylic acid. About 70 plants transformed with the antisense RNA of OsDR3 or RNAi construct were obtained. Some of the transgenic plants displayed higher susceptibility to Xoo than the control. The reduction of resistance was correlated with the decreased expressing level or inhibited expression of OsDR3 as revealed by RT-PCR analysis. These results suggest that OsDR3 gene may positively regulate the processes of resistance against both bacterial blight disease and blast disease in rice.

POSTER



Poster # 165

MOLECULAR MECHANISM OF A NOVEL U-BOX / ARM-REPEAT PROTEIN-REGULATED PROGRAMMED CELL DEATH AND DISEASE RESISTANCE IN RICE

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The rice spotted leaf11 (spl11) mutant was identified from an ethyl methanesulfonate-mutagenized indica cultivar IR68 population and was previously shown to display spontaneous cell death phenotype and enhanced resistance to rice fungal and bacterial pathogens. Here we have isolated Spl11 via a map-based cloning strategy. The isolation of the Spl11 gene was facilitated by the identification of three additional spl11 alleles from an IR64 mutant collection. The predicted SPL11 protein contains both a U-box domain and an armadillo repeat (ARM) domain, which was demonstrated in yeast and mammalian systems to be involved in ubiquitination and protein-protein interactions, respectively. Amino acid sequence comparison indicated that the similarity between SPL11 and other plant U-box-ARM proteins is mostly restricted to the U-box and ARM repeat regions. A single base substitution was detected in spl11, which results in a premature stop codon in the SPL11 protein. Expression analysis indicated that Spl11 is induced in both incompatible and compatible rice-blast interactions. In vitro ubiquitination assay indicated that the SPL11 protein possesses E3 ubiquitin ligase activity that is dependent on an intact U-box domain, suggesting a role of the ubiquitination system in the control of plant cell death and defense. Eight interacting SPL11 proteins have been identified. Characterization of these genes will be presented.

Reference: Zeng LR, Qu S, Bordeos A, Yang C, Baraoidan M, Yan H, Xie Q, Nahm BH, Leung H, and GL Wang. (2004). Spl11, a Negative Regulator of Plant Cell Death and Defense, Encodes a U-Box/ARM Repeat Protein Endowed with E3 Ubiquitin Ligase Activity. Plant Cell, 16(10):2795-808.

POSTER

Poster # 166

THE GENETIC AND PHYSICAL STRUCTURE OF THE MAIZE (ZEA MAYS CV B73) GENOME

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Maize (Zea mays) is one of the most important cereal crops in the world and is now the next target for genome sequencing by the plant community. The maize genome (~2300Mb) is nearly five times bigger than that of rice (~430 Mb) and is rich in repetitive sequences, which comprise approximately 80% of the genome. Due to genome size and repetitive DNA content, scientists are now performing pilot projects to determine the most efficient strategy to sequence the maize genome. Regardless of the approach, a complete sequence-ready physical map of the maize genome will be required. Here we report the sequence-ready physical map of the maize genome that will be of great utility for the maize geneticists and lay the foundation for the maize whole-genome sequencing project.

We generated 292,039 successful agarose and 350,253 HICF DNA fingerprints. Extensive manual editing of the agarose map utilized the HICF contigs, maize-rice synteny, and nearly 2,000 genetic and 13,688 overgo markers. The physical map has been streamlined to 760 contigs, covering 2149 Mb (equal to 93.4% of the 2300 Mb genome). Of the 760 contigs, 411 are genetically anchored with 1918 markers. The anchored contigs cover 1840 Mb, equal to 80.0% of the maize genome. Of the 349 unanchored contigs (13.4% of the genome), 190 of them have less than 10 BAC clones each. The average size of anchored contigs is 4.48 Mb and that of unanchored ones is 0.88 Mb. The longest anchored contig is 22.9 Mb on Chromosome 9 while the longest unanchored contig is 14.3 Mb.

We have used this integrated physical and genetic map to construct a maize genome duplication map, a maize-rice synteny map, and a maize gene distribution map based upon the EST-derived overgo markers and BAC end sequences.

POSTER

Poster # 167

CANDIDATE GENE CHARACTERIZATION AT THE PUP1 LOCUS: A MAJOR QTL INCREASING TOLERANCE TO PHOSPHORUS DEFICIENCY

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Phosphorus (P) deficiency is a major constraint to increasing rice productivity in upland and rainfed lowland environments. Tight binding of P in the soil rather than low total P content is frequently the principal cause for P deficiency. The development of rice cultivars capable of using higher portions of this fixed P is a cost-effective approach to increasing rice yields on P deficient soils. A major QTL for P uptake from P fixing soils (Pup1) had been identified on chromosome 12. The QTL effect was confirmed using a near isogenic line (NIL-Pup1) carrying the positive Pup1 allele from the donor parent Kasalath. P uptake from a P-fixing soil increased 4-fold in NIL-Pup1 compared to Nipponbare, with equally large effects on plant growth and grain yield. Further fine mapping anchored the Pup1 locus to a 220 kb sequence interval spanning 3 BAC clones. The Pup1 interval contains one known and 30 putative genes. To identify candidate genes we performed an RT-PCR analysis using RNA extracted from roots of Nipponbare and NIL-Pup1 that had been grown in nutrient solution at three levels of P supply (high, low, zero). Differential expression between Nipponbare and NIL-Pup1 was detected in several genes but for two candidates, the treatment factor P had an additional effect. Candidate gene 1 was constitutively expressed in Nipponbare but in NIL-Pup1, high expression levels were detected only at zero-P with no expression at high P. The second target gene was only expressed in NIL-Pup1 and its expression level increased with increasing P deficiency. The potential role of both target genes in P uptake is discussed based on a combination of physiological evidence and detailed sequence analysis.

POSTER

Poster # 168

THE ENHANCER TRAP LINES CARRYING GAL4-UAS SYSTEM AS A VALUABLE TOOL FOR RICE FUNCTIONAL GENOMICS

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As a part of our rice functional genomics project, an enhancer trap system carrying a GAL4/VP16 transactivator and a UAS-GFP reporter cassette was employed to generate a rice T-DNA insertional mutant library. We have currently obtained more than 70,000 independent transformants. About 10,000 T1 transformants families have been screened for morphologically conspicuous mutations. Various mutant phenotypes related to plant height, leaf color, heading date, flower and root morphology have been collected, and many of the mutations have been identified as due to T-DNA insertion. Tail-PCR was used to isolate the T-DNA flanking sequences. A rice database containing 5000 flanking sequences has been established and used for reverse genetic research. We carried out GFP assay in different tissues and established the reporter gene expression profiles of 4000 independent T0 transformed plants at vegetative and reproductive stages. Some pattern lines, in which GUS expressed in specific flower tissues obtained earlier, were used for ectopic expression assay of transcription factors , and the results indicated that the GAL4/ VP16-UAS ectopic system is a useful tool to unveil the functions of critical genes, including functions that do not exist in the natural status of the genes (gain-of-functions). These results clearly demonstrated that this system has provided very valuable resources for rice functional genomics studies.

POSTER

Poster # 169

ISOLATION AND INITIAL PROTEOMIC ANALYSIS OF AMYLOPLASTS FROM ESTABLISHMENT OF GAL4-UAS ECTOPIC EXPRESSION SYSTEM IN CHARACTERIZATION OF TRANSCRIPTION FACTORS IN RICE

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During plant growth and development, a large number of genes are spatially and temporally expressed to cope with the requirements on cell differentiation and responses to environment cues. The strategy of ectopic expression of target genes in specific tissue or organs has been widely used to uncover the latent roles of genes in model species such as fly and Arabidopsis. This study aimed to i) establish the GAL4-UAS (upstream activation sequence) ectopic expression system in rice and, ii) characterize the functions of rice transcription factor genes that may not be revealed by gene suppression or overexpression approaches.

The binary GAL4-UAS system essentially consists of two types of transgenic lines, activator lines (driver lines) and effector lines (target lines). The activator construct contains the transactivator GAL/VP16 with minimal promoter and the UAS-GUSplus in a binary T-DNA vector. An insertion of T-DNA at the downstream of a tissue/organ-specific promoter or enhancer will activate the expression of the GAL4/VP16, which in turn will turn on the GUS expression thus to generate a pattern line. Target line was generated by transformation of a construct carrying the UAS cis-element fused to the interested gene and the EGFP reporter gene respectively. By crossing a pattern line and a target line, the target gene can be activated in the hybrid in a pattern as defined by the pattern line.

From screening of about 1000 activator transgenic lines, several pattern lines that displayed GUS expression in flower-specific, anther-specific, stamen-specific, leaf-specific and ubiquitous expression pattern, respectively, were identified. Meanwhile, target lines were generated for 24 transcription factors (belonging to MADS, NAC, MYB and LIM subfamily) with unknown function. So far, 120 crosses were made between pattern lines and target lines. The GFP assays showed that the GFP reporter gene was expressed in the hybrids in tissue/organ-specific manner as expected from the pattern lines. RNA gel blot analysis showed that the ectopic expression of target gene was coincided with the GFP expression. Interestingly, phenotypic changes were also observed for quite a few transcription factors that were ectopically expressed. For an example, ectopic expression of a putative rice MYB gene, with GFP co-expressed, in young leaves of seedlings caused PCD-like cell death and finally led to death of the whole plant. Our results suggest that the GAL4-UAS system has been successfully established in rice and it can be used as a powerful tool to unveil the function of genes in controlling the fundamental processes related to plant growth, differentiation, reproduction and cell death.

POSTER

Poster # 170

RIFGP CDNA MICROARRAY DATABASE

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As part of the Rice Functional Genomics Program of China, we constructed a 10K cDNA microarray (Lan et al. 2004, Plant Mol Biol 54:471-487). To integrate the information, we have developed a database (RIFGP-CDMD) consisting of complete datasets, including the probe sequences, microarray images, raw data, and experiment description. The main goal of this database is to provide different users to download the microarray datasets and search for the expression profiling related to specific genes. The RIFGP-CDMD homepage (http://plantbiol.genetics.ac.cn) is accessed by a free registration and includes four parts: 1.Microarray experiment explorer: rice cDNA microarray data by projects and experimental information; 2.Analysis of cDNA clones: the cDNA information by clone ID and blast sequences against local cDNA database and other databases; 3.Search data by sample information: download the data for specific experimental conditions; 4.Experiment data submission: submit the sample information and raw data for the new experiments. Further integration of the database with additional information like rice proteomics is in progress.

POSTER

Poster # 171

GENOTYPING AND PHENOTYPING THE USDA RICE CORE COLLECTION

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The USDA (United States Department of Agriculture) rice core collection consists of 1,801 entries from 115 countries, representing about 10% of the entirety of 17,395 accessions in the National Small Grains Collection. These are identified in the Germplasm Resources Information Network (GRIN). Nine Oryza species were included with the majority being O. sativa. The core was established by the stratified random sampling method as follows: 1) recording the number of accessions from each country of origin in the whole collection; 2) calculating the logarithm (log) index of the number of accessions from each country; 3) randomly choosing the accessions within each country based on the relative log index, with a minimum of one accession per country; and 4) removing duplications.

Following several years of phenotyping, genotyping was started with 183 publicly available rice microsatellite markers (www.gramene.org). These markers were analyzed using an ABI 3700 automated capillary sequencer with fluorescently-labeled primers. One hundred sixty nine out of the 183 markers, distributed over the rice genome with an average of 14 markers per chromosome and an average genetic distance of 9 cM between markers, have been previously used to evaluate genetic diversity and population structure in U.S. and international rice accessions. Assessment of the core using microsatellite markers will facilitate analysis of genetic relationships and assist in identifying genomic regions that harbor agriculturally important genes. Also, molecular markers related to grain quality descriptors are being analyzed in cooperation with the USDA-Beaumont Research Unit.

The following descriptors have been phenotyped: agronomy - days to flowering, plant height, plant type, panicle type and lodging; morphology - awn type, bran color, grain type, hull color, hull cover, kernel length, kernel width, length/width ratio, rough and brown 1000 kernel weights; and physiology - straighthead. Evaluations are under way for grain quality - alkali/spreading value, amylose, aroma, endosperm type, gelatinization temperature, and protein; pest resistance - blast, sheath blight and stink bugs; and physiology - iron and zinc contents.

All the data from genotyping and phenotyping will be displayed in GRIN (www.ars-grin.gov). With the information, genetic gaps in the entire collection can be identified for further expansion, genetic drift or change when accessions are regenerated can be monitored, and duplicates from regeneration and new introductions can be determined. Thus breeders will find it easier to locate genes or traits of interest. Background information for those accessions containing the desired traits including genetic distances of these lines from commercial cultivars will help in designing effective strategies for transferring the traits.

POSTER

Poster # 172

A RICE EIN2-LIKE GENE MEDIATES ETHYLENE AND ABSCISIC ACID CROSSTALK AND INVERSELY REGULATES DISEASE RESISTANCE AND ABIOTIC STRESS TOLERANCE

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EIN2 is a central component of ethylene signaling pathway in Arabidopsis. In contrast to Arabidopsis that contains a single copy of EIN2, rice appears to have two EIN2-like genes. In this study, a rice EIN2 homologue was isolated and designated as OsEIN2-2. To functionally characterize OsEIN2-2, transgenic suppression lines were generated through RNA interference. Suppression of OsEIN2-2 did not change the ethylene level in the transgenic lines but negatively affected their seed germination, coleoptile elongation and flowering. Suppression of OsEIN2-2 also resulted in reduced sensitivity to ethylene, but hypersensitivity to abscisic acid. In comparison with control plants, the OsEIN2-2 suppression lines exhibited increased susceptibility to fungal (Magnaporthe grisea) and a bacterial (Burkholderia glumae). Interestingly, the same transgenic lines were much more tolerant to drought, salt and cold stress. These results suggest that OsEIN2-2 mediates the cross-talk between ethylene and abscisic acid signaling and inversely regulates disease resistance and abiotic stress tolerance in rice.

POSTER

Poster # 173

ROLE OF OSMADS50 / OSSOC1 IN THE PHOTOPERIODIC REGULATION OF FLOWERING IN RICE.

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The photoperiodic control of flowering is one of the important responses of plants because it is directly related to the success of reproduction. Previously, we reported that the signaling pathway for day-length response is highly conserved between long-day (Arabidopsis: GI, CO and FT) and short-day plant (rice: OsGI, Hd1 and Hd3a), but only the activity of one component (Hd1) is reserved between these two spices (Hayama et al. 2003).

We have isolated a mutant that exhibited late flowering phenotype under natural light conditions by the screening of T-DNA inserted plants. T-DNA was inserted into the K-region (4th intron) of OsMADS50, which is a homolog of Arabidopsis SOC1 / AGL20. Under short day conditions (10 h / 14 h day / night), the mutant flowered at the same time as wild type (ca. 60 d after sowing), while flowering was late (140 d after sowing) under long day (14 h / 10 h day / night) compared to wild type (90 d after sowing). Transgenic plants overexpressing OsMADS50 / OsSOC1 exhibited early flowering phenotype immediately just after plant regeneration from tissue culture. These results indicate that OsMADS50 / OsSOC1 is a positive regulator of flowering in rice. We are currently investigating expression of key genes involved in flowering in rice at various developmental stages to understand the role of the OsMADS50 / OsSOC1 in flowering of rice.

Hayama et al. (2003) Nature 422: 719-722

POSTER

Poster # 174

THE CELL WALL-ASSOCIATED KINASE (OSWAK) GENE FAMILY AND ITS EVOLUTIONARY EXPANSION IN RICE (*ORYZA SATIVA L.*)

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The cell wall-associated kinases (WAKs), one type of receptor-like kinases (RLKs), are believed to play a role in signal transduction between the plant cell wall and its cytoplasm. Here we identified 129 OsWAK gene family members from the genome sequence of japonica cv. Nipponbare, about five times of WAK/WAKLs (26) in Arabidopsis. Of the 129 OsWAKs, eighty-one are intact members containing the extracellular EGF-like domain(s) (EGF_2 and/or EGF_Ca++) and a cytoplasmic protein kinase domain. Twenty-seven members are truncated in the C-terminus, containing only the protein kinase domain(s). The other twenty-one are truncated in the N-terminus and contain only the presumed extracellular regions of an intact OsWAK, but not a kinase domain. Gene structures of OsWAKs are predicted to have varying numbers (~0-5) of introns. Thirty-five members were verified with full-length cDNAs. Fifty-four OsWAKs (35 intact, 7 N-terminal truncated and 12 C-terminal truncated) were shown as being expressed in various rice tissues; some were induced under biotic and abiotic stresses. Using the conserved kinase domain regions, phylogenetic analyses showed that the 107 OsWAKs with kinase domain(s) could be divided into six groups. OsWAK Groups I and II are similar to Groups I and IV of Arabidopsis WAK/WAKLs, respectively, indicating they originated before monocot and dicot divergence. OsWAK Groups III to VI are specific to rice and are believed to have expanded specifically in monocot species. The 129 OsWAKs are encoded on all twelve rice chromosomes. Local gene duplication accounts for most OsWAK gene family expansion in the rice genome.

POSTER

Poster # 175

FUNCTIONAL AND EVOLUTIONAL ANALYSIS OF THE PI2 BLAST RESISTANCE GENE CLUSTER

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The rice blast resistance (R) gene Pi2 mediates gene-for-gene resistance against diverse strains of the fungus Magnaporthe grisea that express the Avr-Pi2 gene. To understand the molecular basis underlying the broad-spectrum resistance to rice blast, we isolated the Pi2 gene by map-based cloning method. A BAC/TAC contig covering the Pi2 region was constructed and sequenced completely. A gene cluster encompassing 9 NBS-LRR-type candidate resistance genes was identified in the Pi2 region. The Pi2 gene was further delimited in to the region from Nbs1-Pi2 to Nbs6-Pi2 by fine mapping. Characterization of susceptible mutants isolated from gammy ray-mutagenized Pi2 plants further narrowed down Nbs4-Pi2 to be the only Pi2 candidate gene, which was confirmed by the gene complementation test. The Pi2 gene encodes a polyprotein containing an NBS and LRR domain and shows constitutive expression. Interestingly, Pi2 consists of two introns with their unique positions, localized before the NBS domain and after the LRR domain, respectively, a feature distinct from most characterized NBS-LRR R genes in rice. Physical mapping and sequence analysis revealed that Pi2 and Pi9 are allelic although they were introgressed from different donor lines. To elucidate the evolutionary mechanism of the Pi2 locus, we completely sequenced the Pi2-allelic region in Nipponbare. Comparative analysis between the resistant and susceptible alleles revealed that the Pi2 loci in the two cultivars is highly conserved. Comparative analysis of more resistant and susceptible Pi2/Pi9 alleles will also be presented.

POSTER

Poster # 176

DEVELOPMENT AND CHARACTERIZATION OF RICE DELETION MUTANTS FOR FUNCTIONAL GENOMICS

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A large mutant population in single genetic background is useful for determining the functions of DNA sequences using both forward and reverse genetics. The objective of this study was to establish 45,000 independent mutant lines using a mid season US rice cultivar, Katy. Katy, released in 1989 from Arkansas, is a long grain with good yield potential and milling quality and contains effective and durable blast resistance genes. Katy was subsequently used as one of the major sources for US cultivar development programs and was chosen for US mutant population development. To date, 6,000 M1 lines induced by ethyl methane sulfonate (EMS), 14,000 M1 lines induced by fast neutrons and 25,000 M1 lines induced by gamma radiation were collected from 2001 to 2004. Preliminary analysis of M2 seedlings revealed defects in chlorophyll synthesis in approximately 4% of EMS M1 derived lines. A total of 10 blast susceptible mutants derived from fast neutrons and 7 lesion mimic mutants (1 from EMS and 6 from fast neutrons) were identified. Progress on advancing and characterization of these mutant lines will be described. All of these lines will be deposited as genetic stocks in the Genetic Stocks-Oryza (GSOR) collection at Dale Bumpers National Rice Research Center.

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WHOLE GENOME ANALYSIS OF RECOMBINATIONAL EVENTS INVOLVING LTR RETROTRANSPOSONS IN RICE *(ORYZA SATIVA L.)*

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In order to study LTR retroelements distribution and phylogeny in *Oryza sativa L.*, 344 PAC and BAC clones sequenced by the Rice Genome Program, were considered to accurately identify a curated set of retrotransposons. 297 complete elements were identified and classified as members of the Ty-3-gypsy family (225 elements) or Ty-1-copia (71 elements) family. Elements were further classified into subfamilies through similarity searches carried out on LTRs. The 297 complete elements were then used to analyse the distribution of partial and complete LTR retroelements in the rice genome using similarity searches on the almost complete *Oryza sativa* (var. Japonica) genome sequence constituted of 12 chromosome pseudomolecules representing almost 358 Mbp: almost 16 % of the genome sequence considered corresponds to LTR retrotransposons. These elements seem to be evenly dispersed across the rice genome. 3800 complete elements were identified; gypsy-like elements are 3.5 times more abundant than the copia ones. When the chromosome pseudomolecules were searched for solo-LTRs, 7452 occurrences were found. The ratio between solo-LTRs and complete retroelements (1.96) as well as the incidence of nested organization of the retroelements (28.5% of the total) suggest that the mechanisms aimed at the counterbalancing of retrotransposition effects could be active in rice genome. The ratio greatly varies (0.125-23.2) according to the different subfamily considered. The analysis of its variation along the chromosomes shows that recombination frequency decreases towards the centromeric regions. A significant positive correlation has been found between the ratio of solo LTRs to complete elements and the average insertion age of each family. In comparison to Arabidopsis, homologous recombination based mechanisms seem to be playing a greater role in reducing genome size in rice than illegitimate recombination based ones. Nevertheless, legacies of this particular kind of recombination mechanism can still be identified in the rice genome in form of incomplete elements: the amount of rice genomic sequence that was not accounted for by either complete elements or by solo-LTRs was 9.2 Mbp out of a total of 57 Mbp matching retrotransposon sequences. The number of incomplete retrotransposon elements was 612 compared to a total number of 3247 of complete elements. The ratio of incomplete to complete elements was higher for copia (0.21) than gypsy (0.18) elements. Solo-LTRs were proportionally more abundant in comparison to entire elements in the gypsy family than in the copia one even though the two families of elements show similar distributions of insertion times (slightly shifted towards younger elements in the gypsy family) as well as along the chromosomes. A different factor from either time or physical location must therefore account for this significant difference. Sequence divergence between the LTRs from each element of a sample of 702 LTR retrotransposons (including those isolated from the 344 BAC/PAC clones) was used to estimate the time of the insertion into the genome: most elements (more than 85%) seem to have inserted less than 5 Myrs ago whereas only a small portion (less than 0.2%) is more than 20 million years old. The sequences of the most conserved retroelements domains (in this case reverse transcriptase and integrase) were used to build phylogenetic trees. The results were then used in comparison to maize: many retroelements subfamilies seem to predate the divergence between the two species and orthologus subfamilies show different relative abundance in the copia but not in the gypsy group. Rice seems to be characterized by an assortment of retrotransposons and a lack of overrepresented families: this feature is in striking contrast with that identified in maize genome.

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