10th ISRFG

INTERNATIONAL SYMPOSIUM ON RICE FUNCTIONAL GENOMICS

November 26 - 29, 2012 Chiang Mai, Thailand

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ORAL PRESENTATION

POSTER PRESENTATION

DRIVING THE NEXT GREEN REVOLUTION

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Welcome Message

Dear Honorable Participants:

It is our pleasure to welcome you to the 10th International Symposium on Rice Functional Genomics (10th ISRFG) in Chiaing Mai, Thailand on November 26-29, 2012. The ISRFG is an international arena for rice scientists to meet and exchange new knowledge on rice research.

The theme of this year's symposium is "Driving the Next Green Revolution". The symposium covers wide areas in rice genomics which include functional discovery, disease resistance, abiotic stress tolerance, planthopper and rice interaction, epigenetics and regulatory RNA, genome expression database and the use of genomic information for breeding-by-design. Projects such as International Oryza Map Alignment Project and Rice Annotation Project are also included.

Thailand is the home of the famous aromatic rice Khao Dawk Mali 105, the number one premium rice exported by Thailand and widely known in countries importing jasmine rice.

We sincerely welcome you to this symposium and to enjoy nature
and Thai culture at Chiang Mai, Thailand.

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OF-05	**Thousands of rice genomes and phenotypes: challenges and opportunities** *McNally, Kenneth; Mauleon, Ramil; Hamilton, Ruaraidh; Dingkuhn, Michael; Leung, Hei; Dobermann, Achim; Zeigler, Robert; Wright, Mark; McCouch, Susan; Li, Zhikang; Li, Jun; Zhang, Guojie; Bo, Wang; Xu, Xun and Zhang, Gengyun*

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OH-03	**Mapping quantitative trait loci conferring palatability and viscosity of rice** *Meng-Chun Tseng; Yong-Pei Wu; Meng-Ying Lin; Hsin-Ya Huang and Yann-Rong Lin*
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L-01

Rice 2020

Qifa Zhang[1], Jiayang Li[2], Yongbiao Xue[2], Bin Han[3] and Xing Wang Deng[4,5]
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Increasing rice production has been considered as an effective strategy for increasing global food production and safeguarding food security. Rice has also become a model for the genomic study of monocotyledon species, we describe a call for an international coordinated effort in rice functional genomics in the form of a project named RICE2020. The mission of the project will be: to determine the function of every gene in the rice genome by the year 2020, to identify functional diversity of alleles for agriculturally useful genes from the primary gene pool of rice, and to apply the findings of functional genomics research to rice genetic improvement and safeguarding food security. Despite the tremendous progress made by rice researchers, there is still a huge gap of knowledge for bridging the genotype and phenotype, which is essential for breeding elite varieties suitable for sustainable agriculture. We thus propose an International Rice Functional Genomics Project (IRFGP), with a goal to determine the function of every gene in the rice genome by the year 2020, to identify functional diversity of alleles for agriculturally useful genes from the primary gene pool, and to apply the findings of functional genomics research to rice crop genetic improvement and beyond. We propose the following objectives for this international effort, with elaboration of specific aims to be achieved.

(1) Development of Enabling Tools and Genetic Resources for an International Community of Scientists to Conduct Functional Genomics Research in Rice Under this objective, we propose three main aims to be achieved. Those are: (1) insertion mutant collections, (2) full length cDNA collections, and (3) artificial micro-RNA (amiRNA) collections.

(2) Assignment of Biological Functions to Every Annotated Gene
We propose two aims for this objective: (1) systematic phenotyping and characterization of the mutants, and (2) systematic characterization of gene families.

(3) Systems-Wide Epigenomes, Gene Expression Profiles and Regulatory Networks
This objective is proposed to include three aims to be achieved. They are: (1) comprehensive cell- or tissue-specific epigenomes and transcriptomes for selected developmental stages, abiotic and/or biotic conditions; (2) identification of regulatory elements based on the epigenetic profiles and transcriptomes; and (3)

systematic characterization of regulatory hierarchy of genome expression, its relationship to epigenomes during development and responses to various environmental changes, and their effects on growth and development.

(4) Global Analyses of the Proteome and Protein–Protein Interactions
We propose two main aims for this objective: (1) tissue-specific proteomes of selected developmental stages and under selected defense and stress conditions; and (2) an experimentally defining comprehensive protein–protein interaction network.

(5) Natural Variation of O. sativa and its Relatives
We propose two main aims for this objective: (1) sequencing a core set of O. sativa strains and its AA-genome relatives; and (2) develop a comprehensive platform for SNP association study to determine the relationship between phenotype and genotype and to identify functional diversity of agriculturally useful genes.

(6) Bioinformatics, Data Management, and Exchange and Sharing of Information
The specific aims here are to create a comprehensive rice annotation database (cRAD) that also provides data-mining platform for high throughput data analysis by individual researchers.

(7) Establishment of the Toolkit for High-Throughput Knowledge-Based Rice Breeding
The ultimate goal of the rice functional genomics research is to realize the ideal situation of 'breeding by design' to breed cultivars to meet the diverse needs of global rice production for high yield, superior quality, multiple resistances and high nutrient-use efficiency. High throughput and low-cost technologies based on the massive sequence information should be developed for breeding applications, most likely as multiple sets of oligonucleotide chips to meet the diverse needs of rice breeding programs, such as indica vs japonica, and inbreds vs hybrids.

L-02

Mutant resources for the functional analysis of the rice genome

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With the completion of the rice genome sequencing project, the next major challenge is the large-scale determination of gene function. Generation of insertion mutant collections has been initiated in the late 90s at the National Institute of Agrobiological Sciences and at the Pohang University of Technology making use of the endogenous Tos17 retroelement and of the T-DNA, respectively. Soon later, several tagging vectors, including T-DNA, Tos17, Ac/Ds, Spm, have been used to generate rice mutant population worldwide. Currently the international collection of the insertion lines is ~ 675,000, and ~ a quarter of them consist of the information of integration site sequences. Less than 20% of the whole population have the phenomics data. In addition, Physical Deletion mutants (γ-rays, neutron) and Chemical Point Mutants (EMS, SA, MNU) are also available. The M2 or M3 DNA samples are used for reverse genetics by TILLING, and a high throughput TILLING service for rice genes are now open to the community. Recently, next-generation sequencing coupled with multidimensional pooling has been successfully used for the identification of rare alleles in this mutant population. Through international efforts, together we provide good resource for gene function elucidation through forward and reverse genetics approaches

Keyword : Mutant resources, functional genomics

L-03

Rice diversity project

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The cultivated rice gene pool possesses an immense amount of genetic diversity, little of which has been harnessed for crop improvement due to inadequate knowledge of the population and haplotype structure as well as the link between genotypes and phenotypes. The Rice Diversity Project has developed a platform that will foster a deeper understanding of the population and haplotype structure of rice and the relationships between SNPs, haplotypes, and traits. One component of this platform is the panel of 2000 purified genetic stocks spanning the range of *Oryza sativa* variety groups and geographic distribution. This panel has been genotyped using a 700K SNP high-density array developed from SNPs discovered by re-sequencing of over 125 rice genomes. The 700K chip enables detection of 1 SNP every 500-600 bp across the genome, and provides sufficient resolution to define chromosomal regions in linkage disequilibrium with traits of interest. Another crucial component of the project is the global team of partners working together to phenotype the panel for a wide range of traits such as blast tolerance, leaf and panicle anatomy, root system architecture, aluminum tolerance, micronutrient concentration, and numerous agronomic traits. Genome-wide association studies demonstrate that both known and novel genes and QTLs are detectable, and that genetic architecture is specific to different subpopulations. This project has created a global research platform that can be expanded rapidly through community participation to enhance the power and resolution of GWAS in rice.

Keyword: genetic diversity, haplotype structure, GWAS

L-04

C4 Rice Project: Redesigning rice photosynthesis

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The demand for food of the growing population continues to increase over the years. Rice being the staple food of most Asian countries has not reached its full potential being a C3 crop. C4 plants on the other hand have a higher photosynthetic capacity and are more efficient in utilizing water and nutrients. Our research is a part of a large multinational consortium tasked with introducing C4 characteristics into rice. This seminar provides an overview of the many approaches employed by the C4 Rice Consortium: namely, metabolic C4 engineering and identification of determinants of leaf anatomy by mutant screens. The aim of the metabolic C4 engineering approach is to generate a two-celled C4 shuttle in rice by expressing the classical enzymes of the NADP-ME C4 cycle in a cell-appropriate manner. The aim is also to restrict RuBisCO and glycine decarboxylase expression to the bundle sheath (BS) cells of rice in a C4-like fashion by specifically down-regulating their expression in rice mesophyll (M) cells. In addition to the changes in biochemistry, two-celled C4 species show a convergence in leaf anatomy that include increased vein density and reduced numbers of M cells between veins. By screening rice activation-tagged lines and loss-of-function sorghum mutants we endeavour to identify genes controlling these key traits. In this paper, we outline the strategy being adopted by the C4 rice consortium to engineer a more efficient photosynthetic pathway into rice. We also summarize related research in this area.

Keyword: C4 photosynthesis, rice, genetic engineering

L-05

The International-*Oryza* Map Alignment Project: A golden path to unlock the genetic diversity hidden within the wild relatives of rice

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The International-*Oryza* Map Alignment Project was organized in 2007 at the ISRFG meeting in Tsukuba, Japan, and has held annual progress report and coordination meetings in conjunction with the ISRFG meetings ever since. The overarching goal of IOMAP is to create and exploit a genus-level comparative genomics platform that can be used to address fundamental and applied questions in evolutionary biology and plant breeding to help solve the 9 billion-people question. The three main research foci of I-OMAP are to: 1) Generate Reference Genome Sequences for all eight AA genome species, and a representative species of the nine other genome types (BB,CC,BBCC,CCDD,EE,FF,GG, KKLL, HHJJ); 2) Generate, Catalogue, and Provided Access to ABC, CSSL, RIL populations for the AA genome species for functional and breeding studies; and 3)Identify collections of naturally occurring populations of the wild *Oryza* species for diversity, conservation, population and evolutionary analyses.

This talk will discuss I-OMAP's progress in sequencing and annotating the collective *Oryza* genome with a special emphasis on four genomes recently completed at the Arizona Genomics Institute (AGI) – *O. glaberrima* [AA], *O. barthii* [AA], *O. punctata* [BB], and *Leersia perrieri* (*Oryza* out group species).

Keyword: IOMAP, wild *Oryza* species, evolutionary biology

L-06

Integrating informatics resources for rice-learning from the international Arabidopsis informatics consortium

Blake Meyers

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Informatics data for rice is increasing in quantity, diversity, and complexity. It is important for the community of rice scientists to integrate these data into a commonly accessible platform that allows the development of a range of applications ("apps") for customized use of these data. The International Arabidopsis Informatics Consortium (IAIC) was initiated following two community-organized workshops held in 2010 to address increasing bioinformatics needs for Arabidopsis data and in response to funding concerns for the community's primary database, TAIR. The goal of this community-led international initiative is to manage the increasing amounts and types of data and to leverage growing resources, knowledge, and collaborations. An Arabidopsis web "portal" will be built on a distributed system of data, tools and resources that would be funded by a variety of sources under an international management and scientific advisory board. It will be dynamic and represent the evolving needs and capacities of the community while reflecting the funding interests of the respective countries. The core will consist of four parts: 1) the Arabidopsis Information Portal (AIP); 2) the "gold-standard" genome annotation; 3) genome/sequence curation of data on gene functions; and 4) stocks and resources databases. Cohesive, cooperative, and long-term international collaboration will be critical to successfully maintain an Arabidopsis database infrastructure that is essential for plant biology research worldwide. The efforts in Arabidopsis may prove informative for the planning of rice informatics resources.

Keyword : International Arabidopsis Informatics Consortium (IAIC)

L-07

Next-generation sequencing

W. Richard McCombie

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10th INTERNATIONAL SYMPOSIUM ON RICE FUNCTIONAL GENOMICS
November 26 - 29, 2012 Chiang Mai Thailand

L-08

A comprehensive map of rice genome variation from large population-scale genome sequencing: next generation sequencing

Bin Han

Chinese Academy of Sciences, China

L-09

Planthopper genomics for alleviating its outbreak and critical damage to rice

Hiroaki Noda

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The brown planthopper (BPH), *Nilaparvata lugens*, is the most serious pest in rice production. It causes hopperburn by heavy infestation and transmits virus diseases. Serious outbreak of BPH was observed in Asian countries in 2005 and has often occurred since then. The outbreaks appear to be due to insecticide resistance of BPH against neonichotinoid insecticides, including imidacloprid, which have been successfully used for planthopper control since 1990's. The insecticide-resistant populations spread by long distance migration from tropical to subtropical regions in Asia. Genome studies will contribute to important problems in BPH as a rice pest: appearance of virulent strains against BPH-resistant rice varieties, appearance of insecticide resistant strains, and rice virus transmission. EST analysis of BPH has been started about 10 years ago and genome sequencing a few years ago using next-generation sequencing technology. Genome size of BPH was about 1.2 Gb and latest genome assembly created about 277,000 contigs and 239,000 scaffolds. Unigenes based on expressed gene sequences were about 80,000 genes; about 23,500 genes showed protein-coding sequences. Genome information and oligomicroarray are used for BPH studies. Imidacloprid Insecticide resistance seems due to elevated detoxification enzyme (P450) activity in the field BPH populations. Genes related to BPH feeding and starvation were also studied using the microarray.

Keyword : planthopper, Nilaparvata, BPH, genome

L-10

Integration of reverse and forward genetics approaches to uncover naturally occurring variations in rice

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Elucidating the association between naturally occurring nucleotide variations and phenotypic changes has been a challenge in plant molecular genetics. Forward genetics approaches such as map-based cloning are powerful strategies to accomplish this; however, it might be difficult to detect all variations, especially those with minor effects on phenotype. To facilitate this task, we have been developing a series of chromosome segment substitution lines (CSSLs) using diverse accessions from Asia as donor lines. Some of the donors were selected on the basis of their phenotypic performance for traits of economic interest. In addition, 10 accessions were selected from different varietal groups on the basis of their sequence diversity. Some materials have already been developed, and others will soon be completed. To uncover naturally occurring sequence variations, we have also begun whole-genome sequencing of the donor and recipient lines by using next-generation sequencing. Multiple alignments of those genome sequences may allow us to identify target genes in which candidate functional polymorphisms, such as indels or base changes leading to amino acid substitutions, have occurred. Once we find target genes of interest, we can predict their functions on the basis of gene annotation information and expression profiles (i.e., tissue and developmental-stage specificity. To clarify the phenotypic effects of particular sequence changes, we will compare the morphological and physiological phenotypes of CSSLs containing the target chromosomal region from the donor lines with the phenotypes of the recurrent parent. We may also be able to develop overexpression or RNAi lines to elucidate the functions of target genes. Alternatively, we could select gene disruptant lines for target genes from mutant panels developed with Tos17, T-DNA, etc. Proof of concept of this new approach will be demonstrated with an analysis of flowering time.

Keyword : quantitative trait loci, allele mining, whole genome sequencing, chromosome segment substitution lines

L-11

The 3K Rice Project

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Rice is the most important grain crop and the staple food for more than half of the world population. Supported by both the Chinese government and Melinda & Gates Foundation, CAAS-SIBI, IRRI and BGI jointly launched the "**3k Rice Project**" in 2011. The goal of the project is to reveal the details in genomes of the 3000 core collection accessions that represent ~90% of the total genetic diversity within the primary gene pool of rice. To date, high genome-coverage (~13X) re-sequencing of the 3,024 rice genotypes has been completed which yielded 205G paired-end reads, including 17Tb of high-quality clean reads. Sequence reads were aligned to the rice reference genome (IRGSP v7.0) using the BWA software, resulting in identification of 4,513,484,325 (1,492,555/genotype) single nucleotide polymorphisms (SNPs) genome-wide and 556,027,395 (183,872/genotype) insertions and deletions (indels). These indels ranged from 1-50 bp in length. Currently, the SNP data are under extensive analyses to reveal the population structure of the 3k accessions using software such as the TREE, PCA, STRUCTURE, LD, Polymorphism, GWAS *etc*. Announcements regarding the availabilities of the genome sequence data and seeds of the sequenced germplasm accessions and a call for an international effort for deep mining of the huge amounts of sequence data will be made with an ultimate goal to turn the Rice Genebank into a permanent database and information repertoire of ready-to-use genetic resources accessible to the worldwide rice research community. Strategies of using the sequence data for discovering large numbers of genes conferring resistance to rice bacterial leaf blight and for developing future molecular breeding will also be presented.

L-12

The biogenesis and regulatory roles of plant smallRNAs

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We have been investigating the function and biogenesis of small RNAs in plants, specifically including rice and maize. In plants, heterochromatic siRNAs direct DNA methylation. We examined transgenic rice produced though tissue culture in which the impact on small RNAs and the epigenome (DNA methylation) is poorly understood. Whole genome, single nucleotide resolution maps of DNA methylation in rice lines regenerated from tissue culture showed had significant losses of methylation compared to unregenerated plants. Loss of methylation was largely stable across generations, and was associated with loss of 24-nt siRNAs. Loss of methylation at promoters was associated with deregulated expression of protein-coding genes. Analyses of callus and untransformed plants regenerated from callus indicated that loss of methylation is stochastically induced at the tissue culture step. Hence transgenic rice is not only genetically modified but also epigenetically modified. In separate work, we continue to investigate in rice, maize and other plants, the impact of mutations in small RNA biogenesis genes on miRNAs, heterochromatic siRNAs, and trans-acting (or "phased") secondary siRNAs. As time allows, I will discuss our recent progress in this area.

Keyword : small RNA, epigenetics, regulate

L-13

Development of C4 traits in rice: is non-Kranz anatomy an option?

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A feature of photosynthesis in all plants is fixation of CO_2 into organic matter via Rubisco in the carbon assimilation cycle. A constraint on the process is conditions where CO_2 becomes limiting, which leads to increases in photorespiration due to high temperature, drought, or soil salinity. In response to CO_2 limitations, some plants evolved mechanisms to concentrate CO_2 around Rubisco through a C_4cycle. This requires spatial separation of fixation of atmospheric CO_2 into C_4 acids, and the donation of CO_2 from C_4 acids via decarboxylases, to Rubisco. For many years in terrestrial species this was exclusively associated with Kranz anatomy, which has evolved independently many times with great diversity in structural and biochemical forms. More recently, it was shown that C_4 photosynthesis can occur within individual photosynthetic cells. Two very novel means of accomplishing this evolved in family Chenopodiaceae by spatial development of two cytoplasmic domains which contain dimorphic chloroplasts. Our results provide evidence that one domain is specialized for supporting fixation of atmospheric CO_2 in the C_4 cycle, and the other for accepting CO_2 from decarboxylation of C_4 acids and its assimilation by Rubisco in the C_3 cycle. Further molecular analyses are needed to characterize genetic requirements for its function, and to determine how these unexpected forms of C_4 developed. Emerging information on strategies for accomplishing C_4 has promise for improving the productivity of rice, which lacks a CO_2 concentrating mechanism, and for securing this important crop as a food supply under CO_2 limited conditions predicted with global warming.

Keyword : C4 photosynthesis (Oral Plenary Structural Genomics)

L-14

New engineering targets to simultaneously improve crop water and radiation use efficiencies

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Improving crop water and radiation use efficiencies are major areas of crop breeding research. Various approaches have been independently developed to increase these efficiencies. These approaches have played critical roles for the improved crop yields during the past few decades. Our recent theoretical studies and field tests showed that optimizing leaf chlorophyll content has great potential to dramatically increase water, radiation and even nitrogen use efficiencies. Here I briefly introduce the theoretical background and current field test result of this new principle. I will also briefly discuss the major areas that need to be developed to enable this new engineering principle to be effectively used in crop breeding.

Keyword : photosynthesis, crop yield, water use efficiency

L-15

Decoding the transcriptome diversity for genetic modification of grain-specific traits in rice

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Transcriptome analysis involving five stages of rice seed development highlighted the expression of 19,309 genes. Out of a total of 37,927 genes, 2889 were up-regulated with respect to four vegetative controls, the major ones being signal transduction components and transcription factors. The genes involved in seed development were found to be related to starch and betanidin degradation, gibberellin, brassinosteroid, cytokinin and auxin biosynthesis as also water-deficit inducible. Selected target genes are being analysed for function. Also, 997 transcription factor genes, out of 2193, were differentially expressed in seeds of varieties IR64, Nipponbare, Zhenshan 97 and Minghui 63. A set of 3,096 SNP (single nucleotide polymorphism) loci from coding and regulatory sequences of such 493 transcription factor genes along with SNPs from 22 known seed related genes/QTLs as well as 71 GWAS generated target loci were used for genetic association analysis and bi-parental linkage mapping. Based on structural annotation and correspondence of these SNP loci with the known QTL regions (1,879) for rice grain/yield-related traits and *in silico* candidate gene-based association analysis/linkage disequilibrium (LD) mapping, a combination of 384 SNP loci in 314 genes including 221 transcription factor genes were selected and used for large-scale validation and high-throughput genotyping in natural and mapping populations. A selected set of 50 SNP loci in 48 rice genes, including 12 transcription factor genes showing strong genetic association with three major grain traits, were validated through traditional bi-parental linkage mapping using RIL mapping population. The resultant markers have the potential for genetic enhancement of rice.

Keyword : Association mapping, Gene function, Rice, Transcriptome

L-16

Functional genomics analysis of regulatory genes controlling flowering time

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In order to understand the regulatory networks controlling the flowering signal pathways, we have systemically elucidated a large number of regulatory genes by screening T-DNA insertion mutant population of japonica rice. From this approach, we identified several genes that are important for controlling flowering time. Among them, genes involved in chromatin remodeling play critical roles in modulating expression of major flowering repressors. OsVIL2 is a homolog of Arabidopsis VIN3. Knockout mutants of *OsVIL2* displayed late flowering phenotypes under both short day and long day conditions by repressing *OsLFL1*, a constitutive repressor of the downstream gene *Ehd1*. OsVIL2 forms a complex with OsEMF2b that is a component of polycomb repression complex 2 (PCR2). OsTRX1 is a member of trithorax group proteins that activate target genes by modifying chromatin structure. Knockout mutation in OsTRX1 showed late flowering only under long day condition. We will provide overall regulatory networks of the flowering signal pathway in rice.

Keyword : flowering time, chromatin remodeling, polycomb repression complex, trithorax

L-17

Iron biofortification in rice by the introduction of multiple genes involved in iron nutrition

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Over 2 billion people suffer from iron and zinc deficiencies because their plant-based diets are not a sufficiently rich source of these essential elements. Furthermore, for crops like rice, removal of the outer layers of the grain during polishing essentially removes all of the micronutrients, leaving only the starchy endosperm. Developing crop cultivars with increased micronutrient concentrations in the starchy endosperm, an approach known as biofortification, could offer a promising solution.To address the problem of iron-deficiency anemia, iron-biofortified rice was produced using three transgenic approaches: by enhancing iron storage in grains via expression of the iron storage protein ferritin using endosperm-specific promoters, enhancing iron translocation through overproduction of the natural metal chelator nicotianamine, and enhancing iron flux into the endosperm by means of iron(II)-nicotianamine transporter *OsYSL2* expression under the control of an endosperm-specific promoter and sucrose transporter promoter. Our results indicate that the iron concentration in greenhouse-grown T_2 polished seeds was six fold higher and that in paddy field-grown T_3 polished seeds was 4.4-fold higher than that in non-transgenic seeds, with no defect in yield. Moreover, the transgenic seeds accumulated zinc up to 1.6-times in the field. Our results demonstrate that introduction of multiple iron homeostasis genes is more effective for iron biofortification than the single introduction of individual genes.

Keyword : Iron biofortification, iron nutrition, nicotianamine, *OsYSL2*

L-18

Evolution and phylogenomics of iron regulated genes in plants

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Iron is involved in many metabolic processes, such as respiration and photosynthesis, therefore being an essential element for plant development. In rice, iron levels can be deficient or toxic when scarce or excessive amounts, respectively, depending on cultivation system and type of soil. However, in both cases yield reductions can be detected, hurting the crop. The analysis of genome structure and function on regions containing iron homeostasis genes can aid the better understanding of this metal role in rice. A phylogenetic analysis of five metal homeostasis gene families (*NAS, NRAMP, YSL, FRO* and *IRT*) selected in Mono and Dicots, Gymnosperms and Bryophytes was performed. The homologous genes were found using known iron homeostasis gene sequences of *Oryza sativa*, *Arabidopsis thaliana* and *Physcomitrella patens* as queries. The phylogeny was constructed using bioinfomatics tools. A total of 243 gene sequences for 30 plant species were found. The divergence time analysis, as well as patterns of cis regulatory elements in gene families related to iron homeostasis are discussed.

Keyword : iron, evolution, abiotic stress

L-19

Photoprotection as a target for crop improvement

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Absorbed solar energy may be defined as excessive when it exceeds the capacity of photosynthesis to utilize it for assimilation. Although excess light is potentially harmful, plants have a plethora of mechanisms that 'manage' the excess absorbed energy on a molecular level in a way that does not result in photo-oxidative stress. 'Photoprotection' is a broad term that can be used to cover mechanisms that prevent light energy from inducing damage via the generation of high levels of reactive oxygen species (ROS). Although ROS are important signaling molecules in plants, they can have deleterious effects on photosynthesis and other leaf processes that ultimately will reduce growth and plant fitness. An exciting possibility is that manipulating photoprotective pathways is a means to enhance both stress resistance and photosynthetic productivity of crop plants and this is especially important in tropical rice, which is often exposed to high irradiance levels. Work with genetic models has resulted in important advances in understanding the underlying mechanisms and genetic basis of photoprotection. However there may be a balance between the need for photoprotection to limit damage on the one hand and enhancing productivity on the other. There is a clear need to discover traits that confer photoprotective qualities but that do not impact upon productivity in optimal environments. Here I will discuss (1) the genes and mechanisms that are potential targets for manipulation (2) the genomic resources that are being exploited to achieve this and the techniques for selection and (3) integration with agronomy and crop physiology.

Keyword : photoprotection, oxidative stress, biomass, canopy

L-20

Comprehensive detection and mapping of quantitative trait loci improving photosynthetic capacity in high-yielding rice

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In crop physiology studies, yield is divided into three major conceptual components: sink size, source strength, and translocation. Among these components, QTLs have been identified mostly for sink size, because of its relative ease of measurement. Increasing yield potential requires replacing and combining these alleles by marker-assisted selection (MAS). However, in many instances, rice with increased sink size does not attain the expected yield because of a lack of grain filling. Unlike the relatively straightforward methods that can be used to assess sink-size traits, the methods for the physiological evaluation of source and translocation traits are difficult to apply to genetic analysis. To understand the genetic basis of source traits such as photosynthetic capacity as accurately as possible, we developed reciprocal chromosomal segment substitution lines and backcross inbred lines, both of which are beneficial for genetic dissection of complex traits, by using 'Takanari', an *indica* cultivar with high photosynthetic capacity, and 'Koshihikari', a standard *japonica* cultivar. We then devised several methods for the evaluation of photosynthesis-related traits with the aid of technological innovations that facilitate field-scale measurements, and we applied them to the developed populations. Moreover, we used MAS to measure the effects of combining QTLs involved in photosynthesis. We will present our current progress toward this challenge and discuss the prospects for designing rice with an improved sink–source balance.

Keywords: QTL, yield, photosynthesis

L-21

SUB1A's role is survival of submergence and other stresses

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Elongation of aerial tissue is a common response to submergence in rice, enabling to outgrow a slow rising flood. However, this energy-consuming response can result in depletion of energy reserves under prolonged complete submergence. *SUBMERGENCE 1A* (*SUB1A*) restricts underwater elongation of aerial tissue through suppression of carbohydrate consumption and amino acid accumulation, avoiding energy starvation during submergence. Using this quiescence survival strategy, genotypes carrying highly submergence-inducible *SUB1A* can endure complete submergence for 14-16 d. Submerged plants are suddenly exposed to oxygen upon de-submergence, causing to cellular damage by oxidative stress. Reoxygenation also induces leaf desiccation even when the soil contains sufficient water. Besides submergence endurance,*SUB1A* confers tolerance to oxidative stress and dehydration through augmentation of reactive oxygen species detoxification and induction of drought tolerance regulators and their downstream components. Thus, *SUB1A* is the master regulator of tolerance to submergence, oxidative stress, and dehydration, all of which sequentially occur in the natural progression of a single flood event. Recent studies revealed that *SUB1A*also enhances recovery from prolonged darkness. Genotypes containing *SUB1A* dampens dark-induced chlorophyll degradation and carbohydrate catabolism by restricted ethylene production and reduced responsiveness to jasmonate and salicylic acid. *SUB1A*-mediated mechanism underlying postponement of leaf senescence can aid in prompt recovery from submergence and dehydration.

Keyword : Submergence, oxidative stress, dehydration, senescence, SUB1A

L-22

Xanthomonas oryzae Tal effectors as tools to identify disease resistance in rice

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Genomes of the rice xylem and mesophyll pathogens *Xanthomonas oryzae* pv. oryzae (Xoo) and pv. oryzicola (Xoc) encode numerous secreted transcription factors called TAL effectors. Based on studies in a few rice varieties, some TAL effectors contribute to virulence by activating corresponding host susceptibility genes, and some activate disease resistance genes. The roles of *X. oryzae* TAL effectors in diverse rice backgrounds, however, are poorly understood. We expressed a set of Xoo TAL effectors that promote infection by activating *SWEET* sucrose transporter genes in the TAL effector-deficient *X. oryzae* strain X11-5A, and assessed the induced phenotypes in 21 rice varieties. Xoo TAL effectors enhanced X11-5A virulence on most varieties, but to varying extents depending on the effector and variety. *SWEET* genes were activated in all tested varieties, but increased virulence did not correlate with activation level. When expressed in an Xoc strain, *SWEET* activators also enhanced Xoc virulence on Nipponbare, however, Xoc TAL effectors did not alter *X. oryzae* X11-5A virulence. Our results suggest that *SWEET*-targeting TAL effectors contribute broadly and without tissue specificity to virulence in rice and their function is affected by host differences besides target sequences. Furthermore, we establish the utility of the TAL-deficient strain of *X. oryzae* X11-5A for characterizing individual TAL effectors in rice.

Keyword : *Xanthomonas oryzae*, rice, bacterial blight, TAL effectors

L-23

Strategies for breeding rice for multiple stress tolerance without yield penalty

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The expected world population may grow over 9 billion by 2050. Rice is the single major food source to more than 50% of the world population, and increase in rice yield would significantly ease the pressure on world food production. Abiotic stresses, such as drought, extreme temperatures and high salinity, are major environmental factors that limit plant growth and productivity worldwide. The current global climate changes tend to shift weather to more extreme variations further aggravating the world crop productivity that is already plateaued. However, the breeding of crops more tolerant to various abiotic stresses yet maintaining yield potentials remains an important and challenging task, as it is difficult to improve rice tolerance to abiotic stresses due to complex crosstalk of a network of genes. Consequently, new strategies for breeding rice with multiple stress tolerance while maintaining high productivity has been an important subject of research. We have designed a synthetic composite promoter with minimal background activity while conferring spatial and temporal regulation of stress tolerance protein expression in rice under stress conditions. We have also been successful in altering plant architectures by manipulating GA levels in rice. Both approaches enhance tolerance to various abiotic stresses, water use efficiency, and grain yield in transgenic rice in field grown conditions.

L-24

A rice gene regulates multiple agronomic traits: bacterial resistance, plant height, and plant strength

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Disease resistance, plant height, and plant strength are important agronomic traits for crop improvement. Here we report that a gene, *Xa38(t)*, contributes to the regulation of these traits in rice. *Xa38(t)* confers race-specific qualitative resistance to *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), which causes the devastating bacterial blight disease of rice worldwide. *Xa38(t)* also positively regulates plant strength. Plant carrying *Xa38(t)* showed increased breaking force for both leaf and stem tissues. This gene negatively regulates plant height. Plant carrying *Xa38(t)* was approximately 10 cm shorter than the plant carrying recessive *xa38(t)* at adult stage. The molecular mechanisms of *Xa38(t)*-regulated physiological processes will be discussed.

Keyword: bacterial blight; disease resistance; plant height; plant strength

OA-01

The wild species of *Oryza* are valuable resources for rice improvement

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The genus *Oryza* has two cultivated species (*O. sativa* and *O. glaberrima*) and 22 wild species. They belong to three different gene pools such as primary, secondary and tertiary with 11 genomes. The cultivated species have inherited a small number of traits which are adaptable during the process of domestication. However, there are numerous economically important traits/genes hidden in the wild species of different genomes. The traits are broad-spectrum resistance to diseases and insects, tolerance to major abiotic stresses, resistance to environmental stresses, traits for enhancing yield potential and nutritional value. Several genes for biotic stresses resistance from wild species are identified and transferred into cultivated rice. Interspecific hybrids are produced from all genomes and monosomic alien addition lines are developed by using special methods from nine different genomes to transfer novel resistance genes into cultivated rice. Several cloned resistance genes of distantly related wild species have revealed unique functional characteristics and are of high value for rice improvement.

OA-02

Application of phylogenomics and evolution: New perennial wild rice in Australia

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Wild rice is one of targets to find novel genes or alleles, which are capable to apply rice breeding with whole genome sequences. We have found two alternative perennial types in Australia. Both perennial types have been fully sequenced for ctDNA and mtDNA. Then, the two perennials are found to carry *O. meridionalis*-type (m-type) genomes. However, several nuclear markers suggested that there are rufipogon-type (r-type) and m-type perennials. Newly developed cytoplasmic markers were applied to screen de-novo collection which could be accessed and compared for their life styles under in-situ condition. Then, all accessions including either annuals or the alternative perennials carried m-type cytoplasmic genomes. Core collection stocked in National Institute of Genetics, Japan was also screened the polymorphism. Then, all Asian accessions belonging to *O. rufipogon* were diverged from Australian accessions. Only accessions had been collected in Australia carried m-type plastid genome, which have been regarded as *O. rufipogon* compared to *O. meridionalis* until today. None of another perennial type has been recognized in the core collection. In addition, some polymorphisms found among the de novo accessions were shared with either population in Australian. Thus, they had belonged to a single population in the past. Reproductive barrier could be found among Asian/Australian rufipogon, m-type perennial, and *O. meridinoalis*. We proposed that perennial types were evolved in parallel between Asia and Australia. The past *O. rufipogon* type perennials had dispersed later than other types. The new perennial type would evolve from incipient species with *O. meridinoalis*.

Keyword : Phylogenomics, Evolution

OA-03

CC genome psuedomolecule construction for resolving species diversification

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In the genus *Oryza*, one of the major genome complexes, the *O. officinalis* complex, contains three diploid CC genome species. Three species, *O. eichingeri*, *O. officinalis*, and *O. rhizomatis,* grow in Asia and Africa and have differing genome sizes. Among them *O. officinalis* has the middle genome size, at about 570 Mb, and the widest habitat among CC genome species. We have been constructing pseudomolecules of *O. officinalis* by using Illumina short reads and have covered 438 Mb with about 56.000 scaffolds on the AGI BAC contigs, although these scaffolds have 40 Mb of N arrays. The scaffolds were constructed by assembling 210 bp and 350bp PE (paired-end) reads together with 2.4kb, 6 kb and 8 kb MP (mate pair) sequences. We plan to incorporate short sequences from 700-800bp PE and 40 kb fosmid fragments to cover more genome sequence. After building an *O. officinalis* draft genome, we will expand our efforts to make the two other CC genome species pseudomolecules. This will aid our understanding of evolutionary relationships among diploid CC genomes as well as those with tetraploid BBCC and CCDD genome species.

Keyword : *Oryza officinalis*, CC genome, psuedomolecule, genome assembling

OA-04

Comparative analysis of the AA-genomes species of *Oryza* provides insights into the plant speciation

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Comparative and evolutionary genomic analyses in a extensively phylogenetic framework, comparing to the findings between two genome analyses of Asian cultivated rice *Oryza sativa* (*indica* and *japonica*), are able to greatly enhance our understanding of the nature and genetic basis of plant speciation in general which has largely unsolved so far. Here, the draft genome assemblies of the seven AA-genome *Oryza* species, *O. nivara*, *O. rufipogon*, *O. glaberrima*, *O. barthii*, *O. glumaepatula*, *O. longistaminata* and *O. meridionalis*, are presented for the first time. We show how patterns and divergent rates of sequence difference across them can brighten evolutionary processes of speciation on a genomic scale. Our analyses reveal that structural evolution has played a major role in restructuring these rice genomes and thus made contribution to their speciation, evidenced by different patterns of rearrangements, segmental duplications and gene family turnover. We surprisingly found that TEs have played a major role in divergently accelerating gene evolution along different lineages. Despite noteworthy similarities among these *Oryza* species, we documented numerous putatively non-neutral changes in protein-coding genes and non-coding RNA genes, which may verify to trigger differences in the ecological adaptation of these diverse species in different continents of Asia, America, Africa and Australia. Overall, the resources and analyses undoubtedly offer new opportunities in evolutionary genomics, insights into rapid recent speciation, and a valuable database of functional variation for sustainable utilization of rice germplasm resources and conservation efforts of wild rice.

OA-05

IOMAP: Oryza genome sequencing update

We have 15 genomes that have been assigned/claimed as part of the I-OMAP and here they are:

1) O. glaberrima [AA], *O. barthii* [AA], *O. punctata* [BB], *L. perrieri* [*Oryza* outgroup] – R.Wing

2) *O. glumaepatula* [AA] – A. Oliveira

3) *O. longistaminata* [AA] – W. Wang

4) *O. meridionalis* [AA] – O. Panaud, R. Henry

5) *O. nivara* [AA] – Y. Hsing [AA]

6) *O. rufipogon* [AA] – B. Han, N. Kurata

7) *O. brachyantha* [FF] – M. Chen

7) *O. officinalis* [CC], *O. rhizomatis* [CC], *O. eichingeri* [CC] – N. Kurata

8) *O. australiensis* [EE] – O. Panaud

9) *O. granulata* [GG] – L. Gao

OB-01

RiceXPro: a database for retrieving gene expression information in rice

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An infrastructure for gene expression information enhances various strategies in plant molecular biology particularly in characterization of gene function. We have developed a gene expression profile database, RiceXPro (http://ricexpro.dna.affrc.go.jp/), to provide more comprehensive information on the transcriptome of rice encompassing entire growth cycle from transplanting until harvesting in the natural field conditions and various experimental conditions such as treatment with 6 phytohormones, namely, abscisic acid, gibberellin, auxin, brassinosteroid, cytokinin, and jasmonic acid. The current version of RiceXPro contains 753 gene expression profiles representing 26 data sets, which are currently grouped into three categories, namely, 'field/development' with 572 data corresponding to 12 data sets, 'plant hormone' with 143 data corresponding to 13 data sets, and 'cell- and tissue- type' comprising of 38 microarray data. The search options from each data set retrieve expression information of a gene/genes in a data set representing a specific condition by using keyword search, chromosome search, analysis tools and/or heatmap option. In addition, we have incorporated an interface for a global approach in searching an overall view of the gene expression profiles from multiple data sets within each category. Furthermore, we have also added a BLAST search function that enables users to explore expression profile of a gene/genes with similarity to a query sequence. Therefore, RiceXPro can be used to survey the gene expression signature of rice in sufficient depth and to gain insights on gene function of other cereal crops.

Keyword : gene expression, database

OB-02

RiceFREND: a database of gene coexpression networks in rice

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Gene coexpression network analysis can be used as a powerful approach for identification of gene functions. We have constructed a gene coexpression database of rice, RiceFREND (http://ricefrend.dna.affrc.go.jp/), to provide a platform for more accurate prediction of gene function based on similarity of expression across a wide range of biological conditions. The coexpression analysis of 27,201 genes was based on 815 microarray data representing tissues/organs at different developmental stages, mature organs throughout the growth from transplanting until harvesting in the field, and plant hormone treatment conditions. The database is provided with two search options, namely, 'single guide gene search' and 'multiple guide gene search' to efficiently retrieve information on coexpressed genes. The resulting gene coexpression network is provided in user-friendly web interfaces that include a list of coexpressed genes with various annotations and links to related databases, and gene network viewers such as HyperTree, Cytoscape Web or Graphviz to facilitate visualization and more efficient interpretation of coexpression data. In addition, analysis tools for identification of enriched GO terms and cis-elements can be used for better prediction of biological functions associated with the coexpressed genes. These features allow users to clarify gene functions and gene regulatory networks that could lead to a more thorough understanding of many complex agronomic traits.

Keyword : database expression, coexpression-network, gene-function

OB-03

The rice oligonucleotide array database (ROAD) for an atlas of rice gene expression and its application for functional genomic analysis

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Microarray technologies facilitate high-throughput gene expression analysis. However, the diversity of platforms for rice gene expression analysis hinders efficient analysis. Tools to broadly integrate microarray data from different platforms are needed. In this study, we developed the Rice Oligonucleotide Array Database (ROAD, http://www.ricearray.org) to explore gene expression across 1,867 publicly available rice microarray hybridizations. The ROAD's user-friendly web interface and variety of visualization tools facilitate the extraction of gene expression profiles using gene and microarray element identifications. The ROAD supports meta-analysis of genes expressed in different tissues and at developmental stages. Co-expression analysis tool provides information on co-regulation between genes under general, abiotic and biotic stress conditions. Additionally, functional analysis tools, such as Gene Ontology and KEGG (Kyoto Encyclopedia of Genes and Genomes) Orthology, are embedded in the ROAD. These tools facilitate the identification of meaningful biological patterns in a list of query genes. The Rice Oligonucleotide Array Database provides comprehensive gene expression profiles for all rice genes, and will be a useful resource for researchers of rice and other grass species. In the last, we will show several application of this database for functional genomic analysis.

Keyword : Rice Oligonucleotide Array Database, Gene expression analysis, Meta-analysis, Co-expression, GO enrichment analysis

OB-04

OryzaExpress for rice Omics information resources-A new statistical method for gene expression network analysis

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The large-scale gene expression data allow us the definitive classification of genes based on the gene expression profiles. However, the current method such as hierarchical clustering for a large-scale dataset requires long calculation time and large-scale computer system. To detect gene sets showing similar expression profiles in short calculation time with general computational circumstances, we have developed a statistical method based on correspondence analysis (CA). This permits us to quickly classify genes in personal computers and workstations even for large-scale data. We have also developed a GUI software package "CA Plot Viewer" which users can easily apply to their own expression data. Gene expression network (GEN) is a powerful tool to simultaneously grasp gene sets (gene modules) showing similar expression profiles. A gene set (gene module) provides important clues for understanding the biological function of the gene module and mechanisms of gene expression regulation. Applying our developed method against rice microarray data (NCBI GEO), we classified genes according to similarities in expression profiles. In the current method for GEN construction, Pearson correlation coefficient (PCC) has been widely used as an index to evaluate similarities in expression profiles for gene pairs. Yet, the calculation of PCCs for all gene pairs requires large amounts of both time and computer resources. By using the new method, we constructed rice GENs and updated the integrated database OryzaExpress (http://bioinf.mind.meiji.ac.jp/OryzaExpress/). It assists us to browse GENs and provide principal omics annotations with a graphical and interactive viewer.

Keyword : bioinformatics database gene expression network

OB-05

Gramene: A community resource for comparative rice and plant genomics

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The Gramene database (http://www.gramene.org) is a curated data resource for comparative genome and functional analysis for rice and other major crop plant species. By integrating genomic sequences and annotations of genes, proteins, genetic and physical maps, germplasm, large scale genetic diversity data sets, and metabolic pathways, the database provides a widely popular online portal for conducting comparative plant biology research. For rice we host the sequenced genomes of *O. sativa* cvs Nipponbare and 93-11 and *O. brachyantha* (a wild relative of cultivated rice). We also host the chr-3 short arm assemblies for several wild rice relatives, contributed by the Oryza Genome Evolution (OGE) project. The genome section provides features like genome alignments, phylogenetic gene trees and variation diversity datasets from the OGE and other international initiatives. RiceCyc, a rice metabolic pathways database allows expression data analysis. Gramene uses ontologies (controlled vocabularies), such as the Gene (GO) and Plant (PO) Ontologies. In the presentation we will demonstrate how researchers can use Gramene to facilitate their research projects not just by browsing and searching but also by uploading their own datasets (visible privately) on various genomics and pathway tools. We will also discuss rice gene expression datasets from salt tolerance experiments and showcase the use of Gramene. All data in Gramene is publicly available, and all code is open source. Gramene is supported by NSF awards (IOS# 0703908 and 1127112) and works closely with EBI-Ensembl Genomes, IRGSP, OGE, and EBI-ATLAS projects.

Keyword : functional genomics, metabolic network, rice genome, systems biology, genetic diversity

OB-06

TRIM: How to use a tagged mutant population with phenomics information

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With the completion of the rice genome sequencing project, the next major challenge is the large-scale determination of gene function. The detail phenomics analysis is the key for functional genomics. Several tagging vectors, including T-DNA, Tos17, Ac/Ds, Spm, have been used to generate rice mutant population worldwide. Here in Taiwan, we have constructed the Taiwan Rice Insertional Mutant population (TRIM, http://trim.sinica.edu.tw/home), which consist of about 100,000 tagged lines. I will use this population as example to demonstrate how to use the flanking sequence and phenomics database to pick up lines with interests.By T-DNA insertional mutagenesis approach, we have generated a rice mutant population containing promoter trap and gene activation/knockout lines using a japonica rice cultivar Tainung 67 (TN67). About 60% of them consist of known integration sites and 80% with phenomics records. This resource is available to all international researchers. During the presentation, I will explain how to use the resource efficiently.

Keyword : phenomics, mutant population, flanking sequence

OC-01

Mapping of virulence-associated gene in the brown planthopper

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In this study, we developed new SSR and SNP markers and constructed the first genetic linkage map for the major rice insect pest, brown planthopper (BPH, *Nilaparvata lugens*). The linkage map was constructed by integrating linkage data from two backcross populations derived from three inbred BPH strains. The consensus map consists of 474 SSRs, 43 SNPs, and 1 STS, for a total of 518 markers in 17 linkage groups. To identify a virulence-associated gene against resistance gene *BPH26*, 81 females offspring of the F_2 population derived from a cross between an avirulent strain (IBS89) and a virulent strain (IBS10) were used to map the gene. QTL analysis of honeydew excretion and appearance of an abdomen reveled that the virulence-associated gene was located between SSR markers NLGS649 and NLGS2284 in LG14. This study constitutes a gateway for future genetic analyses of virulence in BPH.

Keyword : *Nilaparvata lugens*; brown planthopper; genetic linkage map; QTL analysis; virulence-associated gene

OC-02

The novel *Terpene synthase* strengthening brown planthopper resistance regulated by candidate *Bph3* genes in rice

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One of the major brown planthopper (BPH) resistance gene; *Bph3*; of rice cultivar Rathu heenati (RH) was mapped on rice chromosome 6 and used in breeding program of the Thai jasmine "KDML105 (KD)" rice however the introgression lines carrying only this region shows less resistance than their donor and the phenotypic-selected introgression lines (PSILs) suggesting that there are other resistant-related gene(s) that co-transferred to KD in the phenotyping selection process. Genomic hybridization of microarray between KD and PSILs identified *terpene synthase* (TPS) gene on chromosome 4 contain natural variation that affect expression of the gene in KD and RH. Sequence comparison suggested 2 SNP at 5' upstream of the gene controls the expression at transcription level and 2 SNP at exon 5 controls the gene function at translation level. Phylogenetic analysis of amino acid sequences suggested that this newly identified TPS is *sesquiterpene synthase*. Comparison of volatile compounds accumulation between KD and RH infested by BPH revealed that RH accumulated higher level of trans-β-farnesene; β-ionone; δ-cardinene; calacorene; germacrean B; and nerolidol. Positional cloning of *Bph3* gene on rice chromosome 6 delimited the candidate gene(s) into 87.9-kb region contains 18 annotated genes including 12 retrotransposon; 4 expressed proteins; 1 hypothetical protein and 1 NBS-LRR disease resistance gene. One expressed protein showed differentially expression between KD and PSIL; reduced by BPH feeding in KD but not affected in PSIL. Roles of Bph3 candidate region and TPS gene were investigated by antixenosis; antibiosis and tolerance mechanisms. It was found that TPS play role in early BPH infestation period while Bph3 play more important role in tolerance of the prolong BPH infested rice.

Keyword : brown planthopper; terpene synthase; sesquiterpene; insect-resistance

OC-03

Resistance to rice *tungro bacilliform* virus is associated with a dominant locus in chromosome 4

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Rice tungro disease (RTD) is a destructive disease of rice in tropical Asia. RTD is caused by rice tungro spherical virus (RTSV) and rice tungro bacilliform virus (RTBV). Indonesian cultivar Utri Merah was found to be resistant to both RTSV and RTBV. TW16 is an RTBV-resistant advanced backcross inbred line derived from the cross between Utri Merah and RTBV-susceptible cultivar Taichung Native1 (TN1). To genetically characterize the RTBV resistance originating from Utri Merah; the association between RTBV resistance and genotypic variation was examined using mapping populations developed between TW16 and TN1. F_1 plants between TW16 and TN1 showed RTBV resistance comparable to that of Utri Merah; and of TW16; indicating that RTBV resistance is a dominant trait. Phenotypes of F_3 plants derived from a single RTBV-resistant F_2plant segregated into 1:3 ratio for susceptible and resistance; suggesting that RTBV resistance in the F_2 plant is controlled by a single dominant locus. Examination for 1536 single nucleotide polymorphisms (SNPs) in the genomes of Utri Merah; TN1 and TW16 indicated the presence of 4 introgression regions from Utri Merah in chromosomes 4; 6; 7 and 12 of TW16. Genotyping for the 4 introgression regions in segregating F_2 and F_3 plants showed that the introgression from 28 to 33Mb of chromosome 4 co-segregates with RTBV resistance. Further association analysis narrowed the RTBV resistance locus within an approximately 100kb region from 31.3 to 31.4Mb of chromosome 4.

Keyword : Rice Tungro disease (RTD); rice *tungro bacilliform* virus (RTBV); rice tungro spherical virus (RTSV); Finemapping

OD-01

Signaling processes and disease protection during the arbuscular mycorrhizal symbiosis in rice

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Arbuscular mycorrhizal (AM) fungi are obligate ubiquitous symbionts found in natural and agroecosystems. Root colonization by AM fungi improves the uptake of water and mineral nutrients in the host plant, mainly phosphorus and nitrogen, in exchange of photosynthetically fixed carbon. The mycorrhizal symbiosis has also been associated with tolerance to abiotic stress and/or resistance to pathogen infection (mainly root pathogens). One important group of plants that associates with AM fungi, and benefits from this interaction, is legumes. Although rice is also a host for AM fungi, limited information is currently available on the relevance of the AM symbiosis in rice plants. We investigated the AM-induced alterations in gene expression in rice plants, focusing on the relevance of the plant defense response during the symbiotic interaction. Colonization by the AM fungus *Rhizophagus irregularis* (formerly named *Glomus intraradices*) induces the expression of genes that are typically associated with the host defense response against pathogen infection in root tissues, supporting that AM fungi have evolved the capacity to circumvent defense mechanisms that are controlled by the rice's immune system. The systemic induction of genes that play a regulatory role in the rice defense response occurs in mycorrhizal plants which are accompanied by an enhanced resistance to the foliar pathogen *Magnaporthe oryzae*, the causal agent of the rice blast disease. This study provides new insights into the molecular mechanisms involved in the mycorrhiza-induced pathogen resistance in rice plants.

Keyword : Key words: arbuscular mycorrhiza, defense response, Magnaporthe oryzae

OD-02

Involvement of mitogen activated protein kinase kinase 6 in UV induced transcripts accumulation of genes of phytoalexin biosynthesis in rice

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Ultra violet radiation leads to accumulation of phytoalexins (PA) in rice (*Oryza sativa*) which are typically accumulated when the plants are infected with rice blast pathogen *Magnaporthe oryzae*. Although extensive works have been done in elucidating phytoalexin biosynthesis, UV stress signal transduction leading to accumulations of rice phytoalexin is largely unknown. Here, the involvement of mitogen activated protein kinase (MAPK) cascade in UV induced regulation of genes of phytoalexin biosynthesis has been shown in rice. UV induced activation of MAPK and expression of PA biosynthesis genes were shown to be inhibited with staurosporin and MAPK inhibitors. Transcript regulation studies and kinase assays indicated involvement of OsMKK6 and possibly OsMPK3 in the process. Transgenic rice overexpressing constitutive active *OsMKK6^{EE}* exhibited higher expression of PA biosynthesis genes upon UV stress and also infection of *M. oryzae*. Expression of defense and phenylpropanoid biosynthesis marker genes *OsPR1* and *OsPAL1*, respectively, showed increased expression upon blast infection but not UV elicitation in transgenic lines. bZIP Transcription factor, OsTGAP1 exhibited higher transcripts in *OsMKK6^{EE}* overexpressing plants and indicated to be interacting with MAP kinase. These results suggest a key role of OsMKK6 in producing UV responses leading to upregulation of genes of PA biosynthesis in rice.

Keyword : MAPK, MAPKK, *Magnaporthe oryzae*, phytoalexins, UV

OD-03

Identification of novel quantitative trait loci associated with African strains of *Xanthomonas oryzae* pv. oryzae resistance in rice

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Previous work highlighted differences between Asian and African *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) strains, differences that are also reflected in the race and genome structure. The reference Recombinant Inbred Lines (RIL) mapping population derived from the cross between IR64 and Azucena was used to investigate resistance to African Xoo. Resistance to Xoo strains was assessed on the entire population under greenhouse conditions. Five QTLs for resistance against African Xoo were located on chromosomes 1, 7, 9, 10 and 11. Loci on chromosomes 1, 7, 9, 10 and 11 explained as much as 13, 37, 13, 11 and 15% of resistance variation respectively. The major recessive QTL 7 which controlled 37% of the phenotypic variance is new and specific to African *Xoo* races A2 and A3. Resistance to various Asian Xoo strains has been previously described and Xa genes and QTLs identified or mapped in rice. Using existing information together with the novel QTLs identified here we mapped all known QTLs and Xa genes onto the reference *O. sativa* subs japonica (var. Nipponbare) physical map. Identification of candidate genes for one of the major QTL (7) is undergoing by integrating comprehensive data for rice, including genome sequence, SNP information and genome expression data.

Keyword : BLB, resistance, genomics, *X.oryzae*

OE-01

Development and evaluation of rice transformed with genes for C4 photosynthesis

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Introducing C_4 photosynthesis system into rice could increase its maximum yield potential by 50% through more efficient use of solar energy. Genetic engineering appears to be a logical approach to introduce characteristics of the C_4pathway into rice because major traits associated with C_4 photosynthesis are absent from rice and its related genera. To gain insights into the extent to which a two-celled version of C_4 photosynthesis can be placed into rice, we are using *Agrobacterium*mediated transformation to insert genes encoding C_4 enzymes individually into IR64. In addition, we are investigating the impact of down-regulating endogenous rice genes such as *OsGDCH* (encoding the H subunit of glycine decarboxylase) in mesophyll cells. Various molecular analyses are used to confirm stable integration and expression of the C_4 genes in rice. The number of transgenes integrated is determined by DNA blots, abundance of transcripts by real time quantitative PCR and RNA blotting, and the quantification of accumulated protein by immunoblots. Transgenic rice plants in which we have successfully overexpressed C_4 *Phosphoenolpyruvate Carboxylase, Pyruvate, orthophosphate dikinase* and *NADP-Malate Dehydrogenase* genes from maize are being further analyzed for appropriate traits. We also produced transgenic lines with knockdown of *OsGDCH* transcripts. To engineer an efficient C_4 pathway in rice, these enzymes and other intermediates have to be facilitated by transporters necessary for organelle metabolite exchange. Therefore, genes encoding C_4 transporters such as *2-oxoglutarate/malate*, *Dicarboxylate transporter 1*, *Dicarboxylate transporter 2* and*Phosphoenolpyruvate phosphate translocator* have been transformed. Plants with stable transport protein expression will be crossed with those expressing the core C_4 genes.

Keyword : C4 rice, photosynthesis, transformation, transporter, yield potential

OE-02

An *in silico* metabolic modeling and analysis of the photorespiratory pathway in rice

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Background and aim: Photorespiration, a highly wasteful process of energy dissipation depresses the productivity of C3 plants like rice under dry and hot conditions. Under such conditions, the intracellular concentration of O_2 increases relative to CO_2, and thus, the affinity of RuBisCO to O_2 increases instead of CO_2 leading to carbon loss/reduce productivity. Thus, the study of the relationship between the rates of photosynthesis, photorespiration and productivity is currently a promising strategy to improve rice production. We aimed to study this intricate behavior of rice through metabolic modeling and *in silico* simulation and analysis.

Methods: To achieve this objective, we first reconstructed central metabolic model of rice having 600 unique genes, 482 reactions and 261 unique metabolites, localized into compartments such as: cytosol, plastid and mitochondrion. Simulation was performed using Flux Balance Analysis approach by maximizing leaf tissue biomass at fixed photon uptake with unrestricted CO_2 supply and water. Analysis was designed by varying the ratio of RuBisCO.

Results: Under high photorespiration/severe water stress conditions (RuBisCO ratio < 2), the Calvin cycle did not show any appreciable differences in fluxes between the normal and drought-stressed condition. Mitochondrial respiration increased intensely to support the biomass growth. Significant increase in fluxes was observed for glyoxylate cycle, folate metabolism and aspartate biosynthesis. Malate transporters played crucial role in exporting the excess NADPH from GOGAT cycle in plastid and importing into mitochondrion for glycine oxidation.

Conclusions: The prediction of the classic photorespiratory pathway can contribute in achieving system-level analysis to reveal hidden mechanism of photorespiration.

Key words: Rice, photorespiration, Flux Balance Analysis, RuBisCO

OE-03

Monocotyledonous crop improvement by transgenic expression of dicotyledonous Pattern Recognition Receptor(s)

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All major crop species are monocots and our understanding of their immune system lags behind of what we know about their dicotyledonous counterparts. It is important to test if our knowledge of the dicot immune system is also applicable to monocots and if we can exploit this knowledge to improve crop disease management. Here I will present very promising data on the transgenic expression of EFR, a dicotyledonous pattern recognition receptor (PRR) restricted to the Brassicaceae family, in the monocot staple crop, rice. EFR recognizes a conserved domain of one of the most abundant bacterial proteins called elongation factor-Tu (EF-Tu, Zipfel et al. 2006). Stable transgenic rice lines expressing EFR detect elf18, the minimal active epitope of EF-TU, as measured by multiple read-outs such as defense gene expression and ROS-burst. Importantly, rice lines expressing EFR also become resistant to several distinct virulent isolates of *Xanthomonas oryzae* pv. *oryzae*. In coming months we will test if this resistance response is also observed when infecting EFR-expressing rice plants with other bacterial pathogens. To our knowledge this is the first report of the functional application of a dicotyledonous immune receptor in an important monocotyledonous crop such as rice. Overall the presented data clearly demonstrates that the knowledge gained on the dicot immune system can be used to improve the immune response of important monocotyledonous crop species.

Keyword : immunity, crop improvement, pattern recognition receptor

OE-04

Role of a novel ABA/stress regulated highly proline-rich glycoprotein in mediating rice root growth

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Water deficit stress or ABA treatment induces the expression of a family of four genes, *OsRePRP*, in rice roots, which encode two classes of novel proline-rich glycoproteins with highly repetitive PX_1PX_2 motifs. Potential homologues of *RePRP* are only found in monocots and their functions are virtually unknown. OsRePRP are heavily glycosylated with arabinose and glucose on multiple hydroxyproline residues converted from proline. It is significantly different from arabinogalactan proteins (AGP) with glycan chains composed with arabinose and galactose. The signal peptide and proline-rich domain of OsRePRP are necessary for the plasma membrane localization of these proteins in rice, onion and barley. In rice, ABA treatment increases their expression level and also alters their expression pattern to be primarily in root meristematic region and elongation zone. Over-expression of *OsRePRP2* in transgenic rice reduces root cell elongation in the absence of ABA/stress, similar to the effect of ABA in wild type roots. Moreover, RNAi knock-down lines for both *OsRePRP1* and *OsRePRP2* are less sensitive to ABA treatment in root growth reduction. These observations indicate that OsRePRP proteins are necessary and sufficient for ABA/stress regulation of root growth. It is suggested that OsRePRP1 and OsRePRP2 are functional redundant suppressors for root cell expansion, probably through their interactions with cell wall components near the plasma membrane. The effect of ABA/stress on rice root growth is likely via the action of these novel OsRePRPs.

Keyword : root growth

OE-05

Gene network involved in rice root development and tolerance to abiotic stresses

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The ultimate objective of our team is to identify targets for rice improvement under abiotic constraints by modifying gene networks involved in root development. In this aim, we are combining several approaches at various scales, from the whole root system (architecture) to specific root tissues (anatomy). Indeed, rice displays a large variation in root architecture and anatomy that reflects adaptation to cropping systems andcontributes to plant performance under variable environmental conditions. The fibrous architecture of the rice root system is composed of a large number of crown roots bearing large and small lateral roots. Being able to affect this architecture could contribute to enhance rice amenability under adverse environments. In the same manner, the radial anatomy of rice roots typifies semiaquatic plants with specialized tissues that permit root growth under flooding conditions. Concentric layers of cells arranged around the central cylinder form, from the outside in, the epidermis and the ground tissue made of four tissues (exodermis, sclerenchyma, cortex and endodermis). Each of these tissues plays a specific role in the adaptive response to the availability of nutrients and water. Again, deciphering gene networks underlying meristem and tissue organization will permit to act on these signaling pathways for the sustainable establishment of the rice crop under stress. Our strategy makes use of a range of techniques including QTLs and association mapping, cell imaging, functional genomics and genetic engineering. We will illustrate some of these approaches based on genetic diversity of rice varieties or candidate genes involved in root development.

Keyword : root, development, abiotic stresses

OE-06

African rice domestication associated to alterations of smallRNA transcriptome

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Panicle structure is a key morphological trait related to the yield, as it affects the number of seeds per panicle. Rice domestication constitutes a model system to understand the evolution of morphological traits and associated genomic features, such as the modification of the panicle branching complexity, associated to changes in the pattern and timing of branching. Panicle architecture varies between AA-genome rice species with highly branched panicles in domesticated species (*Oryza sativa* and *O. glaberrima*) as opposed to the poorly branched panicles in their wild relatives species (*O. rufipogon* and *O. barthii* respectively*).* Our objective is to elucidate the impact of domestication on the Molecular Regulatory Networks (MRNs) related to panicle branching and to understand the dynamic of these MRNs in the context of panicle branching diversity observed within species. In this context, we conducted a comparative study between the two African species, *O. glaberrima* and *O. barthii*, analyzing by NGS the small RNA expression at the branching stage of panicle development. Only a very small set of microRNAs are significantly differentially expressed at this developmental stage between the two species. The microRNAs *miR2118* and *miR2275,*known to regulate the production of phased siRNAs during *O. sativa* panicle development, are significantly more expressed at the branching stage in the African wild species compared to the domesticated one, as well as the associated phased siRNA-generating loci. This study suggests an alteration of the panicle-associated small-RNA transcriptome following domestication of African rice. The results obtained so far will be presented.

Keyword : panicle, evo-devo, smallRNAs, phased siRNAs

OE-07

Genome re-sequencing of rice by NGS to accelerate our researches on its natural variations

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Understanding the natural variations in rice is of great benefit to its breeding program for improving yield, quality and sustainability with available adaptation to diverse environments. To accelerate our analysis of rice natural variations by providing basic genomic resources to link sequence mutations of genes with the physiological and morphological traits, genomes from the multiple accessions of rice cultivars grown world-wide, including 17 varieties that have been used for the construction of chromosome segment substitution lines (CSSLs) as well as BAC libraries, were re-sequenced (>40x coverage) using the short-read type of next-generation sequencers (NGS), GAIIx and HiSeq2000. Long-read type of NGS, FLX 454 was also applied for the genome re-sequencing of two rice varieties, Kasalath (aus rice) and Nava (indica rice) from the above accessions, to ~5x coverage for de novo assembly to increase the effectiveness on use and chromosomal mapping of sequence reads beneficially to the SNP or Indel discovery, particularly within each sub-group of O. sativa. A database to show the sequence mutations between the different rice varieties, which was based on the multiple alignments of assembled sequences coming out from the chromosomal mapping of NGS reads to the 'Nipponbare' reference, is now under construction. The presentation will report our current results obtained from the re-sequencing and data analysis of rice genomes which could be expected to play an important role in the future researches to uncover the natural mutations in rice by integrating the reverse- and forward-approaches with use of both the genetic information from those CSSLs and the sequence mutations discovered between their donors. This work is supported by grants from the Ministry of Agriculture, Forestry and Fisheries of Japan (GD2007, QTL5003).

Keyword : genome re-sequencing, next-generation sequencer, natural variation, CSSLs

OE-08

The rice genes for meiotic recombination: *OsDMC1* and *OsRAD51* complemented function in Arabidopsis mutants of *Atdmc1* and *Atrad51*

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The over-expressing of *ProCaMV35S*::*OsDMC1* in the *Arabidopsis thaliana* T-DNA insertion line of *AtDMC1* gave slight functional complementation in the homozygous mutant plants background with 3.5-fold greater seed production (by weight) when compared with the homozygous mutant. On the other hand, the over-expressing of *ProCaMV35S*::*OsRAD51A1* gave reasonable, but not completely functional complementation in a homologous mutant plant background. These results suggest that the *CaMV35S*promoter is able to drive *OsRAD51A1* at a high enough level in the appropriate meiotic phases to permit a reasonable level of complementation for this strand-exchange protein but not for *OsDMC1*. The reasons why the *DMC1* construct did not give more complementation are discussed. Furthermore, major aspects of meiotic HR seems to be conserved between rice and Arabidopsis, especially the fact that *OsRAD51A1* can largely complement the Arabidopsis mutants.

Keyword : Meiotic recombination, Gene Recombination, Rice, Arabidopsis

OE-09

A role of very-long-chain fatty acids in rice shoot development

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Very-long-chain fatty acids (VLCFAs) are the main components of cuticular wax that covers and protects plants from physical and biological stresses. However, the effect of fatty acid composition or the physiological role of VLCFAs on plant development under normal growth conditions is not well understood, although inhibitors of VLCFA biosynthesis are used as herbicides. We analysed loss-of-function mutants of *ONION1* (*ONI1*) and *ONI2*, both of which encode fatty acid elongase catalysing an elongation reaction of a carbon chain of VLCFAs. Detailed analyses of *oni1* and *oni2* suggested that VLCFAs are required for proper differentiation and functionality of L1 (an outermost cell layer) and L1 is required for entire shoot development. We also identified additional mutants that might be associated with biosynthesis of VLCFAs.

Keyword : fatty acid elongase, very-long-chain fatty acid, L1, shoot

OE-10

A killer-protector system regulates both hybrid sterility and segregation distortion in rice

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The Asian cultivated rice (*Oryza sativa* L.) comprises two subspecies (*indica* and *japonica*). Inter-subspecific hybrids are usually sterile. Wide compatibility varieties (WCVs) can produce fertile hybrids when crossed with both *indica* and *japonica* rice. The *S5* locus on chromosome 6 of rice was identified to control hybrid sterility and wide compatibility. We mapped the *S5* locus to a region of five ORFs (ORFs1-5). Transformation of an *indica* allele ORF5 into a *japonica* variety Balilla resulted in female gamete abortion leading to segregation distortion. We identified a killer-protector system at the *S5* locus encoded by three tightly linked genes (ORFs 3-5) that regulates fertility in *indica-japonica* hybrids. During female sporogenesis the joint action of ORF5+ (killer) and ORF4+ (partner) causes endoplasmic reticulum (ER) stress. ORF3+ (protector) can resolve such ER stress producing normal gametes, but ORF3- cannot resolve the ER stress leading to premature programmed cell death (PCD) resulting in embryo-sac abortion. Preferential transmission of ORF3+ gametes causes segregation distortion in the progeny. These results enhanced understanding of the origin and nature of *indica* and *japonica* rice, and also have important implications for rice genetic improvement.

Keyword : Reproductive isolation, inter-subspecific hybrid, S5, femal gamete abortion

OE-11

Molecular control of tapetal cell death during rice male reproductive development

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In higher plants, timely degradation of tapetal cells, the innermost sporophytic cells of the anther wall layer, is a prerequisite for the development of viable pollen grains. We use the tapetal cells as the model to investigate the mechanism underlying programmed cell death in monocot rice (*Oryza sativa*). We identified several key regulators such as *Tapetum Degeneration Retardation* (*TDR*), *PERSISTENT TAPETAL CELL 1* (*PTC1*), *MADS3* and *MICROSPORE AND TAPETUM REGULATOR 1* (*MTR1*) in controlling tapetal cell death. TDR positively triggers by activating the expression of a cysteine protease gene *OsCP1*. *PTC1* controls a conserved switch for programmed tapetal development and degradation, *ptc1* displays necrosis-like uncontrolled tapetal cell death. The rice floral homeotic C-class gene, MADS3, regulates late anther development and pollen formation by maintaining reactive oxygen species (ROS) homeostasis.MTR1 encoding a fasciclin domain protein and is specifically expressed in the male reproductive cells, but it is able to affect both tapetal cell death and microspore development, suggesting it's critical role in coordinating the development of reproductive cells and their adjacent somatic cells. We also investigate the regulatory network of these regulators in programmed male reproductive development in rice.

Keyword : Rice, Anther, Tapetum, Programmed cell death

OE-12

Whole genomic sequencing of cytoplasmic male sterility (CMS) mitochondria derived from *Oryza rufipogon* to uncover CMS-associated genes

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Using next-generation pyrosequencing, we determined whole genomic sequences of RT98-CMS and RT102-CMS mitochondria derived from *Oryza rufipogon*. To identify CMS-associated candidate genes in these mitochondrial genomes, we followed an approach which has been recently employed by Fujii et al (2010) and Bentolila and Stefanov (2012) for identification of candidate genes in CW-CMS and WA-CMS, respectively, and screened the mitochondrial genomes of RT98-CMS and RT102-CMS for the presence of specific *orf*s that were absent in Nipponbare mitochondrial genome, and were chimeric in structure or whose products carry predicted transmembrane domains. We found 6 such *orf*s each in RT98-CMS and RT102-CMS. Using these selected *orf*s as probes, Northern blots of callus mitochondrial RNA were compared between CMS lines and fertility restorer lines. We found one each *orf* which showed different size of transcripts depending on the presence of a *restorer of fertility* (*Rf*) gene in RT98-CMS and RT102-CMS. Because each *orf* is co-transcribed with a known mitochondrial gene and the transcripts are processed in the presence of an *Rf* gene, each *orf* is an excellent candidate for CMS-associated gene for RT98-CMS and RT102-CMS. Our study confirmed the efficacy of this strategy to uncover CMS-associated genes. This study is supported by the Program for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry.

Keyword : Cytoplasmic male sterility, CMS-associated genes, mitochondrial genome, RNA processing

OF-01

The 9th rice annotation project meeting

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With the completion of the *Oryza sativa* (cv. Nipponbare) genome sequencing by the International Rice Genome Sequencing Project (IRGSP) in 2004, The Rice Annotation Project (RAP) was organized with the aim of providing the scientific community with an accurate and timely annotation of the rice genome sequence. One of the major objectives of this project is to facilitate a comprehensive analysis of the genome structure and gene functions of rice on the basis of the annotation. Last year, a high-quality version of the Nipponbare genome assembly, Os-Nipponbare-Reference-IRGSP-1.0, and its annotation was released from the RAP database (RAP-DB: http://rapdb.dna.affrc.go.jp/). In this workshop we would like to present recent progress of the rice genome annotation and analysis, and will discuss future perspective of genome informatics and databases.

Keyword : annotation japonica database

OF-02

Integrated approach to understand rice as a 'Molecular system'

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Understanding rice as a 'molecular system' requires generation and integration of global molecular data. Subsequent to decoding of rice genome, multifaceted approach was initiated to identify and characterize functional elements in rice genome. Global comparative profiling across rice germplasm in terms of variation in the genome, epigenome and transcriptome (including sRNAs) has revealed several agronomically usefull alleles. Similarly, 'TILLING'-based approaches have been adopted to identify important and mutated alleles. Simultaneously, molecular characterization, introgression and pyramiding of important alleles is under progress. One of the most daunting tasks is integration of all the experimental data generated globally. High quality molecular experimental data published on rice biology is not amenable for indexing in database for efficient query and correlation due to its inherent nature. We have undertaken manual curation and organization of the information in a protein-centric manner. Data models have been developed to parse experimental data like RT-PCR, northern analysis, in-situ localization and protein–protein interaction with the help of several ontologies. Web-based data entry portals have been developed to facilitate manual encoding of every experimental detail in the article. Technique-specific interpretation modules are being developed to automatically interpret the data. As a result, published and peer reviewed experimental data can be rapidly searched and correlated across literature. The visualization and search portal is being developed to provide user with an intuitive and flexible search engine. Potential of such exercise in relation to global and Indian data generated would be presented.

Keyword : miRNA, biocuration

OF-03

A database for rice functional genes identified by molecular studies and its applications for rice genomics and breeding

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Recent progress in rice genomics has helped to clarify the associations between genes and phenotypes. In particular, functionally characterized genes (FCGs) in rice, which have been identified by using genetic resources, mutants, and transformants, have provided important information. However, until now an FCG database based on phenotypes has not been established, although we expect that such a database would be useful in the identification of novel genes controlling important traits. To establish an FGC database, we comprehensively searched for articles related to rice functional genomics and extracted information on 702 FCGs, which represent about 1.6% of the predicted loci in the Rice Annotation Project Database. Among the 702 FCGs, 52% (366 genes) had been identified through natural variation and mutant analysis, and 48% (336 genes) were identified by using transgenic plants. These results show that both forward- and reverse-genetics approaches are valuable methods in rice functional genomics. Comparison of genomic locations between these FCGs and the QTLs in the rice QTL database (Q-TARO; http://qtaro.abr.affrc.go.jp/qtaro) revealed that QTL clusters were often co-localized with high-density gene regions, and that different QTLs within these clusters were associated with different genes, suggesting that these QTL clusters are likely to be explained by tightly linked but distinct genes. Information on the FCGs compiled during this study is now available in the Overview of Functionally Characterized Genes in Rice Online database (OGRO) on the Q-TARO website (http://qtaro.abr.affrc.go.jp/ogro). Information on the locations of FCGs could be useful for predicting candidate gene(s) when narrowing down a QTL region.

Keyword : Database, functionally characterized genes, QTL

OF-04

Genome-wide DNA polymorphisms among modern japonica and indica varieties revealed by NGS

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There were 7 modern rice varieties have been sequenced by NGS technology and processed variant calling by open source software. Among limited sequencing data we discovered and published about 8,000 InDel markers, at least 15 bases differentiate base from the reference genome, IRGSP build 5.0. Because these varieties are highly diversified in the pedigree between each other and they shared partial homogenous genetic background with many other modern varieties in Taiwan. We collected application results of these InDel markers for other varieties from our co-workers. There are about 80% reliability for the original varieties, and about 60% for others. On the SNP frequency spectrum of whole genome for all 7 varieties, there were several imply homogeneous with reference genome sequence.
By NGS data we discovered more useful markers from these varieties, not only to improve the uncertain diversity distribution between Nipponbare and 93-11, but also could be applied to other varieties.

Keyword : NGS, SNP, INDEL

OF-05

Thousands of rice genomes and phenotypes: challenges and opportunities

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A major effort under the Global Rice Science Partnership (GRiSP, http://www.grisp.net) is to develop a platform to discover alleles for key traits and deliver these for rice improvement by applying (leveraging) high-throughput technologies. The foundation of this platform is the wealth of rice genetic resources available for high-density genotyping, genome re-sequencing and detailed phenotyping. The Rice SNP consortium (http://www.ricesnp.org) consisting of Cornell, USDA, IRRI and other partners has genotyped a set of 2000 purified genetic stocks by 700 K SNPs. IRRI, CAAS and BGI-Shenzhen have undertaken re-sequencing of 3042 genomes generating over 17 Tb of trimmed reads and an average depth of 14x coverage per genome. Primary analyses of the data have been completed by BGI-Shenzhen. The phenotyping network of GRiSP has designed variety group-specific panels from the 2000 entries used by the Rice SNP consortium. These panels are being phenotyped for drought tolerance traits, yield components, grain quality and disease resistance. The task of annotating, curating, and conducting detailed analyses of the high-density genotypes and assembled genomes and their associated phenotypes is on the horizon. For this, we seek to engage the global community to develop novel computational methods and a federated database system that will allow discovery of genotype/phenotype relationships and facilitate delivery of this information to breeding programs.

Keyword : SNP, genotyping, sequencing, phenotyping

OG-01

Heavy metal transport in rice

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In this presentation, two topics will be covered regarding to the heavy metal transport in rice. One is LCT1, a metal transporter responsible for transporting Cd to grains. LCT1 is expressed in nodes of rice and suppression of LCT1 expression reduced Cd concentration in grains but not in shoots. Physiological study also supports the role of LCT1 in Cd transport to grains in rice. Another topic is Cs transport in rice. We have screened for a mutant with altered metal accumulation in rice grains and found a number of lines with different metal contents. Among them, we found lines with altered Cs contents in rice. After the accidents in the Fukushima Power Plants, Cs contamination in grains is a public concern and we have conducted field experiments to detemine characteristics of Cs transport in rice. These studies in the future will enable us to reduce concentration of toxic metals in grains.

Keyword : cadmium cesium transport

OG-02

A Mn, Fe and Cd uptake transporter, *OsNRAMP5*, in rice

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We have identified a rice Mn, Fe and Cd transporter OsNRAMP5 (Scientific Reports, 2012). *OsNRAMP5* expression was significantly downregulated by the addition of Cd. *OsNRAMP5* expression was observed in the epidermis, exodermis, and outer layers of the cortex as well as in tissues around the xylem. OsNRAMP5 localized to the plasma membrane, where it transported Mn, Fe and Cd. *OsNRAMP5* RNAi (OsNRAMP5i) plants accumulated less Mn. In xylem sap, the Mn and Fe concentration in OsNRAMP5i plants was lower. The growth of OsNRAMP5i plants was comparable to that of WT under control conditions; however, under Mn-deficient conditions, the root and shoot length and SPAD value of OsNRAMP5i was significantly reduced compared to WT. The total or root content of Cd in OsNRAMP5i was less than that of WT, but the suppression of *OsNRAMP5* also increased Cd translocation to shoots, highlighting the importance of this gene for Cd phytoremediation. We developed OsNRAMP5i plants (A5i) using a high Cd accumulating cultivar, Anjana Dhan. *OsNRAMP5* expression in the A5i roots was 33–66% less than that in WT. At 10 μM Cd conditions, the shoot Cd concentration in A5i was up to 4.9 times that in WT and the shoot Cd content in A5i was up to 4.3 times greater than that in WT. This work was supported by the Program for the Promotion of Basic Research Activities for Innovative Biosciences (PROBRAIN) and a grant from the Ministry of Agriculture, Forestry, and Fisheries of Japan (Genomics for Agricultural Innovation, GMB0001).

Keyword : cadmium, manganese, phytoremediation

OG-03

The protein kinase *OsPSTOL1* is the major gene in the *Pup1* QTL

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Phosphorus uptake 1 (*Pup1*) is a major quantitative trait locus (QTL) for phosphorus-deficiency tolerance that was identified about a decade ago in the traditional aus-type rice variety Kasalath. After the function of *Pup1* remained unclear for many years, the major tolerance gene has now been identified coding for a Ser/Thr protein kinase named *Phosphorus Starvation Tolerance 1* (*OsPSTOL1*). This gene is specific to Kasalath and other tolerant rice varieties but absent from intolerant genomes, including the Nipponbare reference genome. *OsPSTOL1* is specifically expressed in root primordia and acts as an enhancer of root growth rather than regulator of directly P-uptake related genes, such as P transporters. The *OsPSTOL1* mediated larger root system enables plants to forage nutrients from a larger soil area thereby enhancing uptake of P, and other nutrients. Expression of root-development and stress-related genes is altered in 35S::*OsPSTOL1* plants suggesting that this gene acts as an important upstream regulator. This is further supported by a germplasm survey using *Pup1*-specific molecular markers that revealed high conservation of *OsPSTOL1* in stress-adapted rice accessions. Analyses of breeding lines developed by marker-assisted backcrossing showed that the presence of *Pup1*/*OsPSTOL1* can significantly enhance yield under medium and severe P deficiency. Introgression of this gene into local rice varieties is expected to have a significant impact on food security, especially for poor farmers which often depend on poor soils or soils with P-fixing properties.

Keyword : QTLs, phosphorus, molecular markers, molecular breeding, stress tolerance genes, protein kinase

OG-04

RNA-Seq analysis reveals basal response of 4 rice genotypes to phosphate starvation

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Rice has developed several morphological and physiological strategies to adapt to phosphate starvation stress. Genotypic variation has been reported for tolerance to phosphate stress. However the molecular bases of these responses, particularly the transcriptional profile of genotypes with variation in tolerance to phosphate stress has not been thoroughly elucidated. We analyzed gene expression profiles in root and shoot at 22 days under Pi-starvation of 4 rice genotypes with different levels of tolerance using the RNA-Seq method. Approx. 254 million sequenced tags were mapped to the IRGSP-1.0 reference genome sequence and an average of about 5,000 transcripts in each genotypes were found to be responsive. Comparative analysis of the four genotypes revealed an overall similarity and distinct differences in the transcriptome resulting from phosphate starvation. We elucidated 1,637 and 1,785 upregulated transcripts among the 4 genotypes as core responsive transcripts in root and shoot, respectively. These responsive transcripts include many well-known phosphate related genes such as *IPS1*, *PAP2*, *SPX1*,*Pht1;1* and *SQD1*. Several dozen core responsive unannotated transcripts among 4 genotypes were also identified. These results suggest that the core responsive transcripts might function in basal response under phosphate starvation. We were also able to identify differences in expression of responsive transcripts among the 4 genotypes. Further analysis of the transcriptome differentiation under phosphate starvation will provide an overview of strategies for tolerance at the molecular level.

This work was supported by a grant from the Ministry of Agriculture, Forestry and Fisheries of Japan (Rice gene expression profiling, RTR-0001)

Keyword : Phosphate starvation, rice, phosphorus, RNA-Seq, transcriptome

OG-05

Key regulators of drought resistance in rice: bZIP transcription factors

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In the ABA signaling-mediated drought responses, transcription factors of a bZIP subfamily known as AREB/ABF/ABI5 have been identified for their crucial regulatory roles in activating the ABA-dependent stress-responsive gene expression in Arabidopsis. However, very few of such key regulators in rice have been characterized. OsbZIP23 and OsbZIP46 are two members of the AREB/ABF/ABI5 subfamily transcription factors in rice. They have high sequence similarity to ABF/AREB transcription factors ABI5 in Arabidopsis. Both genes are strongly but differentially induced by multiple stress treatments and confer tolerance to different stresses. Overexpression of the native *OsbZIP46* gene increased ABA sensitivity but had no positive effect on drought resistance. The activation domain of OsbZIP46 was defined by a series of deletions, and a region (domain D) was identified as having a negative effect on the activation. We produced a constitutive active form of OsbZIP46 (OsbZIP46CA1) with a deletion of domain D. Overexpression of *OsbZIP46CA1* in rice significantly increased tolerance to drought and osmotic stresses. A large number of stress-related genes are differentially affected in the two overexpressors, and many of them predicted to be downstream genes of ABF/AREBs. OsbZIP46 can interact with homologs of SnRK2 protein kinases that phosphorylate ABFs in Arabidopsis. The stress-related genes activated by OsbZIP46CA1 are largely different from those activated by OsbZIP23. We conclude that OsbZIP23 and OsbZIP46 are two key regulators of ABA signaling and drought stress tolerance of rice and have promising value in genetic engineering of drought tolerance.

Keyword : *Oryza*, Drought, Transcriptional regulation, Genetic improvement

OG-06

Identification of quantitative trait loci controlling mesocotyl elongation in rice (*Oryza sativa* L.)

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Mesocotyl length is an important trait for seedling emergence in direct-seeding cultivation in rice. In this study, a backcross inbred line (BIL) population from a cross between Kasalath and Nipponbare was employed to map quantitative trait loci (QTLs) for mesocotyl elongation. A total of 5 QTLs for mesocotyl length were identified on chromosomes 1, 3, 7, 9, and 12 in 2 independent experiments. Two QTLs (*qMel-1* and *qMel-3*) on chromosomes 1 and 3 were consistently detected in both experiments. To fine map the QTLs, a cross was made between 2 chromosome segment substitution lines (CSSL-6 and CSSL-15), each harboring the Kasalath allele across the *qMel-1* and *qMel-3* regions, and an $F_{2:3}$population was developed. Moreover, analysis of two F_3 near-isogenic lines (NILs) derived from thesame cross, indicated that the 2 QTLs act additively in distinct or complementary pathways in controlling mesocotyl elongation. Substitution mapping indicated that the *qMel-1* QTL was located between the 2 SSR markers RM5448 and RM5310, which are 3,799-kb apart, and that the *qMel-3* QTL was located between the 2 SSR markers RM3513 and RM1238, which are 6,964-kb apart. For association mapping, fifty-seven rice accessions from the Rice Diversity Research Set (RDRS) were evaluated for mesocotyl length and were genotyped using SSR markers located near the *qMel-1* and *qMel-3* of chromosome 1 and 3. The results will be discussed.

Keyword : rice (*Oryza sativa* L.), chromosome segment substitution line (CSSL), direct-seeding, mesocotyl elongation, quantitative trait locus (QTL)

OG-07

Overexpression of constitutively active mitogen activated protein kinase kinase 6 enhances tolerance to salt stress in rice

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Abiotic stress including high salinity is the primary cause for reducing rice crop productivity worldwide. The mitogen activated protein kinase (MAPK) cascade play diverse roles in intra and extra cellular stress signalling in plants. A MAPK cascade minimally consists of three kinase: a MKKK (MAPKK kinase), a MKK (MAPK kinase) and a MAPK, which linearlyphosphorylate and activate downstreamkinase in a specific manner. In this study, a MKK from indica rice, OsMKK6 was functionally characterized in salt stress by generating transgenic rice over expressing its constitutively active form. OsMKK6 was made constitutively active ($MKK6^{EE}$) by mutating sereine and threonine at 221 and 227 position to glutamic acid by site directed mutagenesis and transformed in Pusa Basmati-1 by *Agrobacterium* mediated transformation. Several $MKK6^{EE}$ lines generated were confirmed by genomic PCR and northern blot analysis. Microarray analysis of the transgenic plant revealed upregulation of 316 genes and downregulation of 198 genes compared to the wild type. The regulation of some of the genes were validated by quantitative real-time PCR analysis. Transgenic lines showed no apparent physiological difference from the wild types and showed tolerance to salt stress. The transgenic seedlings growing in 200mM NaCl solution showed greater root, shoot length, fresh shoot weight and higher mitogen activated protein kinase activity compared to the wild types suggesting role of OsMKK6 in transducing salt stress signaling. The data can be used further to find the downstream components of OsMKK6 involved in salt stress and revealing complete cascade of MAPK operating during salt stress signaling in *Oryza sativa*.

Keyword : mitogen activated protein kinase kinase, mapkk, salt stress, rice

OG-08

Role of rice *glutathione reductase 3* in salt stress tolerance

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Glutathione reductase (GR) reduces glutathione disulfide to GSH for maintaining the reduced glutathione pool during environmental stresses. However, our understanding of GR activity in salt tolerance is limited. Among three members of the rice glutathione reductase (GR) gene family, the *OsGR3* has been reported to be a non-functional GR due to lack of important domains at N-terminal. In the present study, we identify and functional characterize the *OsGR3* gene. A GC-rich cDNA product of 537 bp was isolated and fused with the truncated *OsGR3* to form the full-length *OsGR3.* Gene expression analysis showed that *OsGR3* is induced by salt stress but not dehydration or heat stress. The GR3-GFP protein fusion is targeted to the chloroplast. Heterologous expression of *OsGR3* in *E. coli* confirms that it can translate to a protein with GR activity. In contrast to the wild type, the *Osgr3* mutant is susceptible to salt and methyl viologen and when transformed with wild type *OsGR3* a normal phenotype can be rescued. Under salt stress, the *Osgr3* mutant presents reduced total GR activity, inhibited growth, a decline in the maximal efficiency of photosystem II, decreased ratio of GSH to oxidized glutathione, increased salt accumulation and a rapid accumulation of H_2O_2 in leaf tissue. In response to salinity, *Osgr3* also shows reduced mRNA expression of genes essential for salt stress tolerance. Thus, loss of OsGR3 causes rice sensitivity to salinity and may contribute to an imbalanced antioxidative defense system, as well as to a decreased expression of salt stress tolerance-associated genes.

Keyword : Rice, Glutathione reductase, Salt tolerance, *OsGR3*

OG-09

Salt- and ABA- inducible *OsGASR1* is involved in salt tolerance

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GAST genes are involved in various roles including stem elongation and flowering. We have identified *OsGASR1* (*Oryza sativa* Gibberellic Acid Stimulated Rice *1*) gene, a member of GAST, which was tagged by *GUS* reporter. T-DNA was inserted at 46-bp upstream of the translation start codon of the *OsGASR1* gene and caused complete loss of the gene expression. In the *osgasr1* null mutant the 2nd leaf blades became longer than those of wild type due to increased cell size. In addition, several *a-amylase* genes were up-regulated, implying that OsGASR1 is a negative regulator of those genes. The *OsGASR1* transcript level was inducible by salt or ABA treatment, but not by GA. Ectopic expression of *OsGASR1* enhanced the salt tolerance of transgenic plants as judged by wilting ratio as well as fresh weight. Because *E. coli* cells producing OsGASR1 protein became more tolerant to SNP than control cells, the enhanced salt tolerance of the transgenic plants is thought due to the ROS resistance. Indeed, the transgenic plants showed less increase of H_2O_2 content when treated salt stress compared to wildtype control plant. Taken together, we suggest that OsGASR1 has important roles in the plant growth as well as the salt stress tolerance.

Keyword : abiotic stress, knockout, OsGASR1, rice, salt tolerance

OG-10

Proteomics approach for deciphering the intricate mechanisms conferring tolerance towards salinity in salt tolerant indica rice

Rai Vandna

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OG-11

BrCIPK1 encoding CBL-interacting protein kinase 1 from *Brassica rapa* regulates abiotic stress responses by increasing proline biosynthesis

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To elucidate the functions of agriculturally useful genes, the Full-length cDNA Over-eXpressor (FOX) gene hunting system was used with FL-cDNA library derived from *Brassica rapa*. We developed and screened 1,150 FOX-rice lines against abiotic stresses. A FOX line, BR37, which showed enhanced tolerance to multiple abiotic stresses was selected. BR37 line has a gene insert of *CIPK1,* which encodes a CBL-interacting protein kinase 1. The *BrCIPK1* gene was composed of 1,982 bp encoding a polypeptide of 502 amino acids. GFP tagged *BrCIPK1* was exclusively observed in the cytoplasmic and peripheral region in plant cell. Analyses of its gene expression showed that its mRNA transcript was differentiallyaccumulated by cold, salinity and drought, indicating its biological roles in the multiple stress response pathways in plants. Furthermore, *Ubi-1*::*BrCIPK1* rice lines showed significant higher biomass, water content and improved proline and free sugar contents compared to the wild type Gopum. *BrCIPK1* gene interacted with rice calcineurin-B-like protein 1, 5 (*OsCBL1, OsCBL5*) suggesting that it is activated by Ca^{2+} bound CBLs in cytosol by calcium spiking and regulates its downstream target proteins in these regions for increasing abiotic stress tolerance. *BrCIPK1* gene may involve in the stress adaptations through the activation of pyrroline-5-carboxylate synthase (*P5CS*) in the proline biosynthetic pathway.The hypothetical mechanism of elevated tolerance on cold, drought and salinity will be presented.

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Keyword : *BrCIPK1, CBL, P5CS*, transgenic, rice

OH-01

Engineering IR64 mega variety to reach 30% of estimated average requirement of dietary iron in polished rice

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High concentration of iron in polished rice could provide a low-cost sustainable strategy to remedy iron deficiency if minimum of 30% of estimated average requirement (EAR) of dietary iron can be obtained. The target of 14 ppm in polished rice was set up by Harvest Plus nutritionists to reach 30 % EAR. Thousands of transgenic events of IR64 mega variety expressing either soybean ferritin, rice ferritin, nicotianamine synthase or yellow stripe-like (YSL) family of protein on various targeted tissues were produced through a high throughput *Agrobacterium*-mediated indica rice transformation platform using different gene constructs. Increase of iron concentration up to 9 folds was obtained by over expressing iron storage protein in combination with iron chelator. The biofortified transgenic events with high iron concentration, single genomic insertion without presence of beyond *Agrobacterium* T-DNA border sequence were evaluated in a confined field trial. Transgenic events showing iron concentration ranging from 9-12 ppm in the polished IR64 rice was obtained. Expression of transgene and a subset of iron homeostasis genes in selected events was analyzed by qPCR

Keyword : Biofortification, iron, ferritin, nicotianamine, indica

OH-02

Identification of elite variety tag SNPs (ETASs) - a new approach unraveling loci underlying crop improvement

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Elite crop varieties usually fix elite alleles that occur with low frequency within non-elite gene pool. Hence a powerful way of dissecting these alleles for desirable agronomical traits is to compare the genomes of elite varieties with the non-elite population, and then identify the elite variety tag alleles. In this study, we sequenced deeply six elite rice varieties and used two large control panels to identify the elite variety tag SNPs (ETASs). We identified many ETASs resulting in either amino acid changes or even gene structure disruption, providing a valuable checklist for quick identification of targeted genes during these elite rice improvement. As an example, we comprehensively characterized one such ETAS of the elite upland rice variety, IRAT104. This site shows a drastic frequency difference between upland and irrigated rice, and the striking selective sweep around it strongly suggests its association with upland rice suitability. Functional analysis also shows that this ETAS probably plays an important role in enhancing upland rice yield during the rice improvement. Our work demonstrates a novel and effective strategy to mine rare agronomically important alleles.

Keyword : Next-generation sequencing; ETASs; crop improvement

OH-03

Mapping quantitative trait loci conferring palatability and viscosity of rice

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Physiochemical properties of milled rice could be employed as a selection index of grain quality in early generations of rice breeding programs, which could reduce labor costs and improve breeding efficiency. A total of 190 RILs derived by *japonica* cultivar TNG 78 crossed with *indica* cultivar TCS 17 were planted in two cropping seasons. Eight physiochemical properties, included palatability (PLS), peak viscosity (PV), hot paste viscosity (HPV), cool paste viscosity (CPV), breakdown viscosity (BDV), setback viscosity (SBV), peak time (PeT), and pasting temperature (PaT), exhibited continuous distribution, and displayed significant positive correlation between any two traits. By using 133 molecular markers covering 12 chromosomes, the linkage map of a total length of 1501.6 cM with an average of 11.4 cM were established for composite interval mapping. A total of 34 QTLs were uncovered with PVE ranged from 1.2% to 78% in the two seasons. Ten-pair QTLs were detected significantly in two environments, and *qHPV6*, *qBDV6*, *qCPV6*, *qSBV6*, *qPKV7*,*qCPV7* and *qSBV7* identified in this study were conservative with previous studies, suggesting these QTLs expressed stably not only in different environments but experimental populations. The QTLs nearby *Wx* displayed the largest effect on traits PLS, HPV, BDV, CPV, SBV and PeT .The effect of six starch biosynthesis-related genes on these 8 traits was performed by a single point analysis, revealing several genes correlated to some traits. The information paves a way for understanding how genes regulating grain quality and marker-assisted selection for promoting grain quality.

Keyword : Palatability analysis, Rapid visco analysis, Quantitative trait loci, Rice grain quality

OH-04

KDML105 chromosome segment substitution lines may pave way in understanding QTL underlying drought resistance and salinity tolerance identified in rainfed lowland ecosystem

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Multiple QTL loci controlling traits associated with drought resistance (DR) were coincidently located in chromosomes 1, 3, 4, 8 and 9 and to understand the effects of gene controlling DR, a population of 139 chromosome segment substitution lines (CSSL) of KDML105 containing small segments of QTL was developed. To test the potential of the population, the CSSLs were evaluated at the reproductive stage for preliminary agronomic performance and yield components under drought stress and results were compared with irrigated condition. The CSSL lines flowered 6 to 7 days earlier than KDML105 and the mean values of yield in the CSSLs particularly those carrying chromosomes 4 and 8 were higher than KDML105 under both conditions. Project initiatives were established to further phenotype the population for drought avoidance and drought tolerance traits which may be useful in identifying genes for DR expressed in the rainfed lowland environments of Thailand. Moreover, the region of drought QTL in chromosome 1 coincides with QTL for salinity tolerance in the same chromosome. The CSSLs were tested for salinity tolerance (ST) and seven lines exhibited higher ST than KDML105 and even produced higher yield. Eight QTL for ST were identified on chromosomes 1, 3, 4, 7, 8, 9, 10 and 12 using the CSSLs and four were located in the same positions of the DR-QTL previously reported. Using the same population, attempts to identify genes involved in salinity tolerance were initiated to determine whether the co-locations of DR and ST QTL indicate a common mechanism for tolerance.

Keyword : drought resistance, chromosome segment substitution lines (CSSL), salinity tolerance, quantitative trait loci

OH-05

Mol ecular breeding of resilient green super rice (GSR) varieties for changing climatic conditions

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In recent years abrupt climatic change patterns resulted in unprecedented floods, drought, salinity, insects and disease outbreaks that threaten food security in Asia. GSR resilience breeding involves introgression breeding and designed QTL pyramiding(DQP) efforts for developing varieties with multiple abiotic and biotic stress tolerance without compromising on grain yield and quality. GSR molecular breeding strategy involving 16 donors and their introgression into a popular adaptive line i.e. Huanghuazhan (HHZ) had allowed us to identify several GSR materials with multiple abiotic and biotic stress tolerant lines. BC_1F_2 populations derived from HHZ (recipient parent) and 16 donors at IRRI were screened simultaneously for three rounds for different abiotic stresses(drought, salinity, submergence, low chemical inputs) and normal irrigated condition that resulted in identification of 845 trait specific introgression lines(ILs) superior over tolerant checks. Adopting this approach, 40 ILs possessing multiple abiotic and biotic stresses tolerances were nominated to the national cooperative testing, Philippines and multi-environment testing (MET) of IRRI. Such GSR cultivars with multiple abiotic stress tolerance can fit well under varied rice ecosystems and changing climatic conditions. GSR IR1-8-S6-S3-Y2(IRIS179-880151, HHZ8-SAL6-SAL3-Y2) cultivar that stood first in stage 2 MET in the Philippines over 5 locations with yield advantage of 10% over NSIC222 under irrigated conditions also possess drought, salinity and moderate submergence tolerance. GSR IR1-8-S6-S3-Y2 has notably higher radiation and nutrient use efficiency.DQP efforts using GSR IR1-8-S6-S3-Y2 at IRRI is on going and was also whole genome sequenced. Success may be attributed to GSR breeding approach to combine appropriate cross tolerance screening and selection techniques. DQP with omic tools would further enhance the grain yield and resilience of GSR.

Keyword : Breeding, Multiple abiotic stress tolerance, Drought, Salinity, Submergence

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Poster Presentation

International Oryza Map Alignment Project 4

PA-01	**CC genome pseudomolecule construction by BAC-supported super scaffolding** *Ohyanagi, H; Kubo, T; Toyoda, A; Fujiyama, A; Fujita, M; Igarashi, K; Yano, K; Goicoechea, JL; Wing, RA; Kurata, N*
PA-02	**Identifying the functional elements in the rice genome by comparisons of multiple *Oryza* genome s** *Yi Liao; Peng Tian; Meijiao Wang; Bo Li; Mingsheng Chen*
PA-03	**Comparative analysis reveals low synteny and gene escape in the rice centromeric regions** *Xuemei Zhang, Mingsheng Chen*
PA-04	**Phylogenetic inference from whole-genome sequence of Taiwan rice cultivars and landraces** *Hour, Ai-Ling; Wei, Fu-Jin; Hsing, Yue-Ie*
PA-05	**Detection of reproductive barriers between Aus and indica rice** *Harushima, Yoshiaki1; Kurata, Nori*
PA-06	**Fine mapping of a QTL for the number of spikelets per panicle by using near-isogenic lines derived from an interspecific cross between *Oryza sativa* and *O.minuta*** *Balkunde, Sangshetty, Le, Hung Linh, Lee, Hyun-Sook, Kim, Dong-Min, Kang, Ju-Won, Ahn, Sang-Nag*
PA-07	**Mapping quantitative trait loci for spikelets per panicle using NILs carrying wild rice (*Oryza rufipogon*) chromosome segments in cultivar background** *Yeo Sang-Min; Yun Yeo-Tae; Kim Hae-Hwang; Ahn Sang-Nag*
PA-08	**Centromere evolution accompanied by inversions and transpositions in the *Oryza* species** *Yi Liao; Xuemei Zhang; Jinfeng Chen; Zetao Bai; Bo Li;Tieyan Liu; Mingsheng Chen*
PA-09	**Sequencing analysis of domestication-related genes of rice landraces collected from Taiwan aboriginal villages** *Yuan-ching Tsai, Yi-fang Chen, Hong-Yin Lin, Ming-hsing Lai, Ai-ling Hour, Yu-chi Chen, Yu-chien, Tseng, Jaw-shu Hsieh, and Yue-ie Hsing*
PA-10	**Introgression pattern on rice domestication history inferred from phylogenomic analysis** *Kumagai, Masahiko; Kawahara, Yoshihiro; Wu, Jianzhong; Itoh, Takeshi*
PA-11	**Indica-japonica subspecies-specific InDel loci: a novel approach for understanding evolutionary relationships in the genus *Oryza*** *Lee, YooJin; Chin, Joong Hyoun;Jiang, Wenzhu; Koh, Hee-Jong*;Thomson, Micheall*

Genome Expression Database

PB-01	**Genome-wide analysis of differentially expressed genes between japonica and indica rice** *Horiuchi, Youko; Harushima, Yoshiaki; Fujisawa Hironori; Ohyanagi Hajime; Fujita Masahiro; Mochizuki Takako; Kurata Nori*
PB-02	**Developmental and physiological functions of rice root revealed by comprehensive transcriptome profiling** *Takehisa, Hinako; Sato, Yutaka; Igarashi, Motoko; Abiko, Tomomi; Antonio, Baltazar; Kamatsuki, Kaori; Minami, Hiroshi; Namiki, Nobukazu; Inukai, Yoshiaki; Nakazono, Mikio; Nagamura, Yoshiaki*
PB-03	**Genome Information Database System for Innovation of Crop and Livestock Production - The current progress of the development of a common infrastructure for genome analysis** *Solovieva Elena; Sunohara Yoshihiro; Nagamura Yoshiaki; Itoh Takeshi; Miyao Akio*
PB-04	**RiceXPro and RiceFREND: Cyclopedic databases of rice gene expression profiling** *Ikawa, Hiroshi; Sato, Yutaka; Takehisa, Hinako; Kamatsuki, Kaori; Minami, Hiroshi; Namiki, Nobukazu; Ohyanagi, Hajime; Sugimoto, Kazuhiko; Antonio, Baltazar; Nagamura, Yoshiaki*
PB-05	**IRRI GALAXY: Enabling bioinformatics for rice scientists** *Mauleon, Ramil P., Thomson, Michael, McNally, Kenneth L., Juanillas, Venice Margaret, Dilla-Ermita, Christine Jade, Leung, Hei*
PB-06	**The Rice South Green hub** *Droc, Gaëtan; Rouard, Mathieu; Larmande, Pierre; Hamelin, Chantal; Guignon, Valentin; Dufayard, Jean-François; Guiderdoni, Emmanuel; Ruiz, Manuel*

Functional Discovery

PC-01	**Improving rice with maize genomic resource, a promising approach** *Wang, Yafei; Liu, Lin; Xiong, Wentao; Peng, Wei; Zeng, Haiyang; Shi, Xue; Luo, Meizhong*
PC-02	**Rice *MPR25* encodes a pentatricopeptide repeat protein and is not only essential for RNA editing of *nad5* transcripts in mitochondria but for chloroplast function** *Toda Takushi, Fujii Sota, Noguchi Ko, Kazama Tomohiko, Toriyama Kinya*
PC-03	**Genetic and phenotypic characterization of giant embryo mutants in rice** Gileung Lee, Yunjoo Lee, Jaebok Cho, Joohyun Lee, Hee-Jong Koh
PC-04	***OsRAD51D*, one of the rice *RAD51* paralogs, participates in reproductive organ formation and seed development in rice *(Oryza sativa L.)*** *Lihua Cui, Hansol Bae,Mi Young Byun, Woo Taek Kim*
PC-05	**Ectopic expression of *OsMADS45* activates the upstream genes *Hd3a* and *RFT1* at an early development stage causing early flowering in rice** *Wang, Jiun-Da; Lo, Shuen-Fang; Li, Yan-Suan; Chen, Po-Ju; Lin, Jenq-Horng and Chen, Liang-Jwu*
PC-06	***FCA/ABAP1* is an important enhancer in ABA signaling in rice seed germination** *Lin, Wan-Chi, Huang, Kuang-ying, Lu, Yung-Yu and Ho, Tuan-hua David*
PC-07	**A large-scale of functional verification of domestication-related genes in rice** *Yesheng Zhang, Fengyi Hu, Wen Wang*
PC-08	**Effect of light intensity on nitrogen uptake and assimilation in rice seedlings** *Wang, Chieh-Ching; Wang, Shu-Jen*
PC-09	**The Rice FUnctional GEnomics (REFUGE) platform: An assessment of 4 years in hosting research projects using rice as a model system for functional analysis** *Delphine Mieulet, Aurore Vernet, Martine Bès, Eve Lorenzini, Pauline Mayonove, Murielle Portefaix, Rosie Sévilla, Pierre Larmande, Alain Ghesquière et Emmanuel Guiderdoni*

PC-10	**Artificial loci for evaluating homologous recombination efficiency in rice** *Delphine Mieulet, Christelle Siré, Aurore Vernet, Laura Chaperon, Fabien Silvestri, Olivier Cotsaftis, Marie Pascale Doutriaux, Fabien Nogué and Emmanuel Guiderdoni*
PC-11	**Involvement of tonoplast-localized sucrose transporter, *OsSUT2*, in long-distance sucrose transport in rice** *Hyun-Bi, Kim; Joon-Seob, Eom; Min-Young, Song; Chiyeol, Kim; Bancha, Mahong; Jong-Seong, Jeon*
PC-12	**Characterization of rice monosaccharide transporters involving the vacuolar sugar transport** *Jung-Il, Cho; Bo, Burla; Dae-Woo, Lee; Hyun-Bi, Kim; Joon-Seob, Eom; Enrico, Martinoia; Junok, Lee; Jong-13Seong, Jeon*
PC-13	**Sucrose transporter expressions regulated by mechanical wounding in rice seedlings** *Dai, Nai-Chiang; Hsiao, Hui-Hsin; Wang, Shu-Jen*
PC-14	**Arbuscular mycorrhizal symbiosis: Its effect on rice Pi uptake** *Catausan, Sheryl; Mattes, Nicolas; Jeong, Kwanho; Heuer, Sigrid*
PC-15	**Engineering a two-celled C4 photosynthetic pathway in rice** *Robert Coe, Shanta Karki, Sarah Covshoff, Michelle Acoba, Efren Bagunu, Alvin Borja, Florence Danila, Albert de Luna, Menard dela Rosa, Leonides Javier, Swati Kamal, Andrea Lazaro, Raquel Libiternos, Gemma Lorenzana, Florencia Montecillo, Bonjovi Nava, Elpidio Panganiban, Irma Quilloy, Czarina Mae Realubit, Juvy Reyes, Isaiah Salazar, Julius Ver Sagun, Joefer Tanfelix, Ronald Tapia, Janine Kaye Vitto, Helen Woodfield, Xiaojia Yin,William Paul Quick, Inez Slamet-Loedin and Julian M. Hibberd*
PC-16	**Overexpression of nicotianamine synthase gene in trangenic rice induces the expression of deoxymugineic acid biosynthesis genes and suppresses the expression of ferritin genes** *Arines, Felichi Mae; Trijatmiko, Kurniawan Rudi; Dueñas, Conrado Jr.; Slamet-Loedin, Inez Hortense*
PC-17	**Molecular analysis of putative flavonoid O-methyltransferases in rice *(Oryza sativa)*** *Hye Lin Park, Kaewta Rattanapisit, Seong Hee Bhoo, Tae-Ryong Hahn and Man-Ho Cho*
PC-18	**Characterization of putative rice *(Oryza sativa)* monolignol β-glucosidases** *Baiya Supaporn; Ekkhara Watsamon; Ketudat-Cairns Mariena; Ketudat-Cairns James*
PC-19	**Natural variations in pseudo-response regulator 37 contribute to rice adaptation to long-day photoperiod at high latitudes** *Koo, Bon-Hyuk; Paek, Nam-Chon*
PC-20	**Phenotypic characterization and functional analysis of Ac/Ds mediated mutant pool in Korean rice germplasm** *Lee, Gang-Seob; Yoon, Ung-Han; Park, Sung Han; Lim, Hae-Min, Yun, Do-Won; Kim, Chang-Kug; Ji, Hyeon-So; Eun, Moo Young; Kim, Tae-Ho*
PC-21	**Forward genetics approach to discover gene controlling amylose content and gelatinization temperature** *Ekawat Chaichumpoo, Siriphat Ruengphayak, Vinitchan Ruanjaichon and Apichart Vanavichit*
PC-22	**The effects of phytochrome interacting ankyrin repeat protein to the *PIF3*phosphorylation** *Jihye Yoo, Kaewta Rattanapisit, Hye Lin Park, Man-Ho Cho, Seong Hee Bhoo and Tae-Ryong Hahn*
PC-23	**Interacting map and minimal domain of rice U-box E3 ubiquitin ligases for specific interactions with E2 enzymes** *Min, Hye Jo; Bae, Hansol; Kim, Woo Taek*
PC-24	**Targeted *SSS4A* gene mutagenesis using Zinc Finger Nucleases *(ZFNs)* in rice genome** *Jung, Yu Jin; Han, Kyoung Hee; Lee, In Hye; Cho, Yong Gu; Kang, Kwon Kyoo*
PC-25	**Molecular characterization of *BrUGE1* encoding UDP-glucose-4-epimerase in rice** *Lee, Hye-Jung; Abdula, Sailila Estilong; Jee, Moo-Geun; Jang, Dae-Won; Nogoy, Franz; Kang, Kwon Kyoo; Cho, Yong-Gu*

PC-26	**Characterization and fine-mapping of a shortened uppermost internode mutant in rice** *Ji Hyeonso; Kim Hakbum; Yun Doh-Won; Yoon Ung-Han; Kim Tae-Ho; Eun Moo-Young; Lee Gang-Seob*
PC-27	**The rice protein kinase *OsPSTOL1* is an enhancer of root growth** *Gamuyao, Rico; Chin, Joong Hyoun; Pariasca-Tanaka, Juan; Pesaresi, Paolo; Catausan, Sheryl; Dalid, Cheryl; Wissuwa, Matthias; Heuer, Sigrid*
PC-28	**Enhanced saccharification of rice straw by senescence-induced expression of cellulose** *Furukawa, Kayoko; Nigorikawa, Mutsumi; Sonoki, Tomonori; Ito, Yukihiro*

Functional Discovery : NGS

PC-29	**Detection of mutations in regenerated rice by whole-genome sequence analysis** *Miyao, Akio; Nakagome, Mariko; Solovieva, Elena; Yuichi, Katayose; Nagamura, Yoshiaki; Itoh, Takeshi; Takahashi, Akira; Hirochika, Hirohiko*
PC-30	**Detection of copy number variations in the rice genome using next-generation sequence data** *Zetao,Bai; Mingsheng, Chen*
PC-31	**RiceVarMap: a comprehensive database of rice genomic variations** *Zhao Hu; Yao Wen; Wang Gongwei; Lian Xingming; Zhang Qifa; Xie Weibo*
PC-32	**Comprehensive analysis of genetic polymorphisms in Korea rice accessions by whole genome resequencing** *In-Seon Jeong, Ung-Han Yoon, Gang-Seob Lee, Hyeon-So Ji, Hyun-Ju Lee, and Tae-Ho Kim*

Functional Discovery : Nutrition

PC-33	**Differences of folate biosynthesis gene expression in colour and colourless husked rice grain** *Pho-am, Sayrung; Malumpong, Chanate and Anukul, Nampeung*
PC-34	**Expression of QTL based and other candidate genes in Madhukar x Swarna RILs with contrasting levels of iron and zinc in unpolished rice grains** *Agarwal Surekha , Kotla Anuradha, Mangrauthia Satendra Kumar, Neelamraju Sarla*
PC-35	**Overexpression of rice *Nicotianamine Synthase2* and soybean *FerritinH1* in transgenic rice alters the expression of endogenous iron acquisition genes** *Francisco, Perigio Jr..; Trijatmiko, Kurniawan Rudi; Duenas, Conrado Jr.; Barry, Gerard; and Slamet-Loedin, Inez Hortense*
PC-36	**Transformation and expression of a gene controlling anthocyanin biosynthesis from *Arabidopsis* in rice** *Inta, Poonsri; Boonklang, Tawatchai; Hermharn, Warunee; Chowpongpang, Srimek; Pongjareankit Saengtong; Sangtong, Varaporn; Sakulsingharoj, Chotipa*
PC-37	**Evaluation of high iron rice expressing *OsYSL2* transporter gene** *Adeva, Cheryl; Trijatmiko, Kurniawan Rudi; Manzanilla, Marina; Borja, Ma. Gina; Torrizo, Lina; Tsakirpaloglou, Nikolaos; Chadha-Mohanty, Prabhjit; Nakanishi, Hiromi; and Slamet-Loedin, Inez*
PC-38	**Reverse and forward genetics approaches to identify mutants conferring high grain Fe density and tolerance to Fe toxicity** *Siriphat Ruengphayak, Vinitchan Ruanjaichon, Supaporn Phromphan, Ekawat Chaichumpoo, Chatree Saensuk, Theerayut Toojinda, Somvong Tragoonrung, Ratchanee Kongkachuichai and Apichart Vanavichit*

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Functional Discovery : Male Sterility

PC-39	**Involvement of ubiquitin-mediated protein degradation machinery during sporophyte-to-gametophyte *(S2G)* transition in rice anthers** *Deveshwar Priyanka, Chawla Mrinalini, Sharma Malini and Kapoor Sanjay*
PC-40	**ETERNAL TAPETUM 1 promotes tapetal cell death during male reproductive development in rice** *Niu Ningning; Liang Wanqi; Yang Xijia; Zhang Dabing*
PC-41	**Microspore and tapetum regulator 1 encodes a secretory fasciclin glycoprotein required for male reproductive development in rice** *Tan, Hexin; Liang, Wanqi; Hu, Jianping; Zhang, Dabing*
PC-42	**Microsatellite Markers: A tool for the validation of true mutants in mutation breeding programme in rice** *Kaung Kyaw, Nay New Nyein Chan*
PC-43	**Analysis of mitochondrial genes causing pollen abortion and restorer of fertility genes in *Oryza rufipogon*** *Kano, Takahiro; Kazama, Tomohiko; Toriyama, Kinya*
PC-44	**A label-free quantitative shotgun protemics analysis of rice pollen development** *Cho, Jaebok; Lee, Wondo: Lee, Joohyun*
PC-45	**Analysis of gene expression in anther of thermo-sensitive genic male sterile rice (TGMS) using cDNA-AFLP** *Pitngam, Keasinee; Ukoskit, Kittipat; Muangprom, Amorntip*
PC-46	***OsMADS32* determinates rice floral organ identity by genetic interacting with *OsMADS1*, *OsMADS58* and *OsMADS13*** *Yun Hu, Zheng Yuan, Wanqi Liang, Dabing Zhang*
PC-47	**Rice *ORMDL* is involved in male sterility by affecting pollen development** *Chueasiri, Chutharat; Chunthong, Ketsuwan; Pitngam, Keasinee; Sangarwut, Numphet; Chakhonkaen, Sriprapai; Suksangpanomrung, Malinee; Muangprom, Amorntip*

Functional Discovery : Root Biology

PC-48	**Transcription factors involved in ammonium assimilation and root growth in rice plants** *Yuan Hu Xuana, Ryza A. Priatamaa, Chang-deok Han*
PC-49	**Formation of the late inner root cortex layer involves *OsSCR2* AND *OsSHR*, gras transcription factors orthologs of *Arabidopsis thaliana AtSCR* and *AtSHR* genes** *Divol, Fanchon; Pauluzzi, Germain; Puig, Jerome; Guiderdoni, Emmanuel; Dievart, Anne; Perin Christophe*
PC-50	**AZORIZ : Specificity of the phytostimulatory cooperation between *Azospirillum lipoferum* and rice** *DROGUE Benoît, PICAULT Nathalie, SANGUIN Hervé, CHAMAM Amel, PRIGENT-COMBARET Claire, MOZAR Michael, Llauro Christel, PANAUD Olivier, WISNIEWSKI-DYÉ Florence*
PC-51	**Preliminary proteomics analysis in young panicle of thermo-sensitive genic male sterility rice lines** *Chakhonkaen, Sriprapai; Ukoskit, Kittipat; Roytrakul, Sittiruk; Paemanee, Atchara; Muangprom, Amorntip*

Genomics of Disease Resistance

PD-01	**Sequence variation of rice blast fungus, *Magnaporthe oryzae*, avirulence genes in Thailand** *Chatchawan Jantasuriyarat; Teerapong Kasetsomboon; Sureeporn Kate-Ngam; Tanee Sriwongchai; Bo Zhou*
PD-02	**The XA21-Associated Kinase 1 *(OsSERK2)* regulates immunity mediated by the *XA21* and *XA3* immune receptors** *Benjamin Schwessinger, Xuewei Chen, Shimin Zuo, Mawsheng Chern, Patrick E. Canlas, Deling Ruan, Pamela C. Ronald*
PD-03	**Potential resource for rice bacterial blight disease resistance in the newly bred lines of Taiwan** *Yang, Jia-ling; Chen,Chun-Wei; Wu, Dong-Hong; Chang, Ran-Sing; Liu, Chu-Yin; Wang, Chang-Sheng; Chen, Kai-Yi; and Chang, Su-Jein*
PD-04	**Identifying a new source of a bacterial blight resistance gene *xa5* in rice variety 'IR62266' and development of a functional marker 'PAxa5', the easy agarose based detection** *Korinsak, Siriporn; Sirithunya, Pattam; Vannavichit, Apichart; and Toojinda, Theerayut*
PD-05	**A defense-responsive gene, *DR153*, negatively regulates rice disease resistance** *Hongtao Cheng; Hongbo Liu; Xianghua Li; Shiping Wang*
PD-06	**Simultaneous quantification of multiple phytohormones and metabolites in rice-bacterium interaction** *Hongbo Liu; Shiping Wang*
PD-07	**Dissection of resistance to brown spot in Multi-parent Advanced Generation Inter-Cross (MAGIC) and mutant populations** *Raghavan Chitra, Vanica Lacorte, Nonoy Bandillo, Michael Thomson, Ramil Mauleon, Rakesh Kumar Singh, Glenn Gregorio, Edilberto Redoña and Hei Leung*
PD-08	**Plant innate immunity mediated by rice *LysM* receptors** *Kaku Hanae, Hayafune Masahiro, Sato Yosuke, Takamizawa Daisuke, Shimizu Takeo, Shinya Tomonori, Shibuya Naoto*
PD-09	**Diversity of cysteine-rich antimicrobial-like peptides in *Oryza sativa* complex species** *Shenton, Matt; Kurata, Nori*
PD-10	**Enhanced resistance to bacterial and fungal pathogens by overexpression of a human cathelicidin antimicrobial peptide (hCAP18/LL-37) in rice** *Lee, In Hye; Jung, Yu Jin; Kang, Kwon Kyoo*

Genomics of Abiotic Stress Tolerance

PE-01	**Expression of OsHSP16.9 gene under salt, dehydration, heat and ABA treatment** *Jung, Yu Jin; Kim, Eun Oh; Cho, Sin Sang ; Kang, Kwon Kyoo*
PE-02	**Partial characterization of rice Class II E3 ubiquitin ligase *OsPUB2* (*Oryza sativa* putative U-box 2), which is involved in abiotic stress responses** *Park, Ki Youl; Bae, Hansol; Byun, Mi Young; Jung, Yejin; Kim, Woo Taek*
PE-03	**Molecular characterization of rice LEA I gene - protein function and gene evolution** *Shih, Ming-Der Shih; Wei, Fu-Jin Wei; Delseny, Michel, Hoekstra, Folkert A.; Hsing, Yue-Ie C.*
PE-04	**Development of transgenic rice lines using FOX-hunting system and identification of genes with abiotic stress tolerance** *Lee, Hye-Jung; Abdula, Sailila Estilong; Jee, Moo-Geun; Jang, Dae-Won; Nino, Marjohn; Kang, Kwon Kyoo; Cho, Yong-Gu*

PE-05	**A modified rice α Amy8 promoter significantly enhances hypoxia-inducible expression of a human epidermal growth factor in transgenic rice seedlings** *Chen, Peng-Wen; Wu, Chung-Shen; Kuo, Wei-Tin; Kao, Chung-Hong; Chang, Chia-Yu; Wu, Hsi-Ten; and Yu, Su-May*
PE-06	**Cloning and characterization of a stress-inducible small GTPase gene from jasmine rice** *Sugunya Pitakrattananukool, Supranee Sitthiphrom, Somboon Anuntalabhochai*
	Genomics of Abiotic Stress Tolerance : Drought
PE-07	**Constitutive activation of transcription factor OsbZIP46 improves drought tolerance in rice** *Ning Tang, Hua Zhang, Xianghua Li, Jinghua Xiao, and Lizhong Xiong*
PE-08	**Mapping traits associated with prolonged drought and heat tolerance in Nagina 22 mutant** *Panigrahy Madhusmita, Poli Yugandhar, D Nageswara Rao , Voleti SR, D Subramanyam, Neelamraju Sarla*
PE-09	**Detection of cis-acting regulation on drought-response genes in rice by allele-specific expression imbalance** *Malabanan, Katrina; Ereful, Nelzo; Lee, David; Swamy, B.P. Mallikarjuna; Kumar,Arvind; Kao, Allen Shumin; Liu, Daisy; Mauleon, Ramil; Leung, Hei*
PE-10	**Identification of a stress-inducible protein phosphatase gene *SPP1* mediating drought resistance through reactive oxygen species scavenging by ABA-independent manner in rice** *You Jun; Xiong Lizhong*
PE-11	**An *OsbZIP23*-interacting protein, BIP4, negatively regulating of ABA signaling and drought stress response in rice** *Ning Tang, Lizhong Xiong*
PE-12	**Molecular and biochemical screening and evaluation of transgenic drought tolerant rice** *Liwanag, Evelyn; Gaudin, Amelie; Laluz, Virginia; Slamet-Loedin, Inez*
PE-13	**A rice Kinesin gene is probably involved in drought stress** *Chanthong, Ketsuwan; Muangprom, Amorntip*
PE-14	**Quantifying the contribution of aquaporins to modulation of rice root hydraulic conductivity under drought** *Grondin A., Cal A.J. and Henry A.*
	Genomics of Abiotic Stress Tolerance : Salt
PE-15	**Genome analysis of salt-tolerant rice** *Udomchalothorn Thanikarn; Comai Luca; Buaboocha Teerapong; Chadchawan Supachitra*
PE-16	**Loss of chloroplastic glutathione reductase 3 enhancing susceptibility to salinity is caused by reduced glutathione redox state and retrograde signals from chloroplast to nucleus** *Wu, Tsung-Meng1; Lin, Wan-Rong1; Hong, Chwan-Yang*
PE17	***OsMLD* encoding MYB-like DNA binding domain increases tolerance to salt stress in rice (*Oryza sativa* L.)** *Lee, Hye-Jung; Abdula, Sailila Estilong; Jee, Moo-Geun; Jang, Dae-Won; Yu, Dal-A; Park, Sung-Han; Yoon, Ung-Han; Kim, Tae-Ho; Cho, Yong-Gu*
PE-18	**Salinity tolerance in rice (*Oryza sativa* L.) during germination** *Risa Nagura; Kosuke Noborio*

PE-19	**Expression of sodium transporter *SKC1* in rice seedling relates to different tolerance response under hydroponic and soil culture salinity stress conditions** *Meechai Siangliw, Asarnha Phatanathara, Risa Nagura, Chatree Saensuk, Theerayut Toojinda and Apichart Vanavichit*
PE-20	**Vacuolar Na+/H+ antiporter overexpression for salt tolerance: a complex regulation in rice** *Sabrina M Elias, Richard Malo, U S Mahzabin Amin, Sudip Biswas, Touhidul Islam, Farhana Nazneen, Saima Shahid, Taslima Haque and Zeba I Seraj*
PE-21	**Expression level of NHX1 gene, a vacuolar Na+/H+ exchanger, Na+ accumulation and physiological changes in rice (*Oryza sativa* L. ssp. indica) responses to salt stress** *Theerawitaya Cattarin, Yamada Nana, Cha-um Suriyan, Takabe Teruhiro, Kirdmanee Chalermpol*
PE-22	**Expression analysis and characterization of rice oligopeptide transport gene (*OsOPT10*) that contributes to salt stress tolerance** *Kang, Kwon Kyoo; Lee, Sel Ki; Jung, Ji Eun; Yang, Hee Kyoung; Lee, Kye Dong; Jung, Yu Jin*
PE-23	**Transcriptional regulations of the genes of starch metabolism and physiological changes in response to salt stress rice (*Oryza sativa* L.) seedlings** *Theerawitaya, Cattarin; Boriboonkaset, Thanaphol; Cha-um, Suriyan; Supaibulwatana, Kanyaratt; Kirdmanee, Chalermpol*
PE-24	**Identification of salt responsive genes in an advanced backcross population from a cross between *Oryza sativa* L. cv. Milyang23 and *O. glaberrima*** *Kim, Dongmin; Kim, Haehwang; Kang, Juwan; Lee, Hyunsook; Ahn, Sangnag*
	Genomics of Abiotic Stress Tolerance : Submergence
PE-25	**QTL mapping and confirmation for tolerance of anaerobic conditions during germination derived from the rice landrace Ma-Zhan (Red)** *Ignacio, John Carlos; Sendon, Pamella Marie; Sanchez, Darlene; Ismail, Abdelbagi; Mackill, David; Septiningsih, Endang*
PE-26	**Characterization of a major QTL for tolerance to anaerobic germination for direct-seeded systems** *Pelayo, Margaret Anne; Kretzschmar, Tobias; Gabunada, Lourd Franz; Abdelbagi, Ismail; Mackill, David; Septiningsih, Endang*
PE-27	**Gene validation of a major QTL for tolerance of anaerobic conditions during germination** *Tobias Kretzschmar, Rudi K.Trijatmiko, Margaret A. Pelayo, Lourd F. Gabunada, Pamella M. D. Sendon, Inez Slamez-Loedin, David J. Mackill, Adelbagi M Ismail, Endang Septiningsih*
PE-28	***Sub1* locus affects differential transcriptome that regulates the energy consumption and recycling under flash-flooding conditions** *Ruanjaichon, Vinitchan; Tragoonrung Somvong; Vanavichit, Apichart*
	Genomics of Abiotic Stress Tolerance : Cold
PE-29	**Comparative transcriptomics analysis of TNG67 and TCN1 rice seedlings with contrast chilling stress responsiveness** *Yang, Yun-wei; Chang, Men-chi*
PE-30	**Toward the understanding of genetic architecture for cold tolerance among the local population in the northern-limit of rice cultivation** *Fujino, Kenji; Takahashi, Junya; Nakada, Akiko; Kuroki, Makoto; Takamure, Itsuro*

Genomics of Abiotic Stress Tolerance : Heat	
PE-31	**Analysis of pollen-specific gene expression and phenotypic observation in several rice varieties subjected to heat stress** *Kim, Sunghan1; Lee, Dongryung1; Lee, Yun-Young1 and Koh, Hee-Jong*
PE-32	**Association analysis of seed longevity in rice (*Oryza sativa* L.) under conventional and high-temperature germination conditions** *Li Gang, Ra Young-Wang, Kwon Soon-Wook, Li Feng-Peng, Ra Won-Hee, and Park Yong-Jin*
Genomics of Abiotic Stress Tolerance : Ion	
PE-33	**Glutamine synthetase (GS) in rice enhances tolerance to cadmium and abiotic stresses** *Lee, Hye-Jung; Abdula, Sailila Estilong; Jee, Moo-Geun; Jang, Dae-Won; Park, Sung-Han; Yoon, Ung-Han; Kim, Tae-Ho; Yong-Gu Cho*
PE-34	**Transgenic rice tolerant to low iron availability in a calcareous soil harboring combination of the mutational reconstructed ferric reductase gene and Fe-deficiency inducible transcription factor, *OsIRO2*** *Erika, Shimochi; Hiroshi, Masuda; Tatsuro, Hamada; Takanori, Kobayashi; Yasuhiro, Ishimaru; Yuko, Ogo; Hiromi, Nakanishi; Naoko, K., Nishizawa*
PE-35	**Characterizing the response of rice to iron excess** *Bashir Khurram, Nakanishi Hiromi, Nishizawa Naoko K.*
PE-36	***OsNRAMP5*-RNAi rice accumulates higher cadmium and contributes to rapid and efficient cadmium extraction from paddy field** *Takahashi,Ryuichi; Ishimaru, Yasuhiro; Shimo, Hugo; Bashir, Khurram; Senoura, Takeshi; Sugimoto, Kazuhiko; Ono, Kazuko; Yano, Masahiro; Nishizawa, K. Naoko; Nakanishi, Hiromi*
PE-37	**Rice plasma membrane intrinsic proteins, are involved in transport and providing tolerance to boron toxicity** *Kundan Kumar; Kareem A. Mosa; Sudesh Chhikara and Om Parkash Dhankher*
PE-38	**Evaluation of ferritin and iron transporter proteins in biofortified rice** *Oliva ,Norman; Trijatmiko, Kurniawan; Adeva, Cheryl; Chadha-Mohanty, Prabhjit; Barry, Gerard; Slamet-Loedin, Inez*
PE-39	**Identification of the chromosomal integration site in rice genome using Zinc Finger Nucleases** *Cantos, Christian; Mulyaningsih, Enung Sri; Francisco, Perigio Jr.; Trijatmiko, Kurniawan Rudi; Slamet-Loedin, Inez and Chadha-Mohanty, Prabhjit*
PE-40	**Varietals development for alleviating impacts of global warming during reproductive and seed production stages : Screening for high temperature tolerant rice mutation lines by spikelet fertility.** *Sulaiman Che-abu, NAT Panichawong, Prisana Rattanametta, Chanate Malumpong, Tiwa Pakoktom, Boonthong Wasuri, Poonpipope Kasemsap and Apichart Vanavichit*
Epigenetics and Regulatory RNA	
PF-01	**RNA-directed DNA methylation is involved in regulating photoperiod-sensitive male sterility in rice** *Ding Jihua ; Shen Jianqiang ; Mao Hailiang ; Xie Weibo; Li Xianghua ; Zhang Qifa*
PF-02	**Structural basis of a plant histone H3 lysine 4 demethylase required for cell division** *Xiangsong, Chen; Qingfeng Chen; Quan, Wang; Faben, Zhang; Zhiyong, Lou; Qifa, Zhang; Dao-Xiu, Zhou*
PF-03	**DNA methylation biomarker of 5-methyltryptophan-resistant rice mutants** *Jung, Yu Jin; Han, Kyoung Hee; Lee, In Hye; Cho, Yong Gu; Kang, Kwon Kyoo*

Breeding-by-design

PG-01	**Simulation of genome structure and power of QTL detection in rice multi-parent advanced intercross lines** *Yamamoto, Eiji; Tanabata, Takanari; Mizobuchi, Ritsuko; Yonemaru, Jun-ichi; Yamamoto, Toshio; Yano, Masahiro*
PG-02	**Heterosis QTLs for grain yield and yield-related traits in indica-japonica crosses of rice** *Chu, Sang-Ho; Jiang, Wenzhu; Chin, Joong Hyoun; Koh, Hee-Jong*
PG-03	**Uncovering the genomic structure of Japanese high--biomass rice cultivars derived from japonica-indica crosses** *Jun-ichi,Yonemaru; Ritsuko, Mizobuchi; Toshio, Yamamoto; Eiji, Yamamoto; Kaworu, Ebana; Hiroshi, Kato; Kazuki, Matasubara; Hideyuki, Hirabayashi; Yoshinobu, Takeuchi; Hiroshi, Tsunematsu; Takuro, Ishii; Hisatoshi, Ohta; Hideo, Maeda; Masahir*
PG-04	**QTL analysis for some agronomic characters in two rice populations derived from wide-compatibility line** *Seo, Jeonghwan; Lee, Yunjoo; Kim, Donggwan; Koh, Hee-Jong*
PG-05	**Yield evaluation of two-line hybrid rice** *Sangarwut, Numphet; Chueasiri, Chutharat; Chunthong, Ketsuwan; Pitnjam, Keasinee; Chakhonkaen, Sriprapai; Janbuathong, Supaporn; Palawisut, Suradet; Sopprasong, Wanwisa; Taprab, Suniyom; Muangprom, Amorntip*
PG-06	**Genetic composition of yield heterosis in an elite rice hybrid** *Zhou,Gang;Chen,Ying;Yao,Wen;Zhang,Chengjun;Xie,Weibo;Hua,jingping;Xing,Yongzhong;Xiao,Jinghua;Zhang,Qifa*
PG-07	**QTL mapping of rice biomass-related traits in recombinant inbred lines from crosses between high-yielding cultivars** *Matsubara, Kazuki; Mizobuchi, Ritsuko; Kobayashi, Nobuya; Tanaka, Jun-ichi; Tsunematsu, Hiroshi; Yamamoto, Eiji; Yonemaru, Jun-ichi; Amari, Masahiro; Matsumura, Osamu; Yamamoto, Toshio; Yano, Masahiro; Kato, Hiroshi*
PG-08	**A yeast two hybrid screening to search for protein that interact with *RF2*, a restorer of fertility of LD-type cytoplasmic male sterility in rice** *Fujii, Shinya; Kazama, Tomohiko; Ito, Yukihiro; Kojima, Soichi; Toriyama, Kinya*
PG-09	**Fine mapping of a quantitative trait loci controlling the number of spikelets per panicle in rice** *Kim, Dong-Min; Kim, Ji-Hoon; Kang, Ju-Won; Lee, Hyun-Sook;, Ahn, Sang-Nag*
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PA-01

CC genome pseudomolecule construction by BAC-supported super scaffolding

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The Genus *Oryza* consists of multiple genome complexes. The *Oryza officinalis* complex is one of the major genome complexes which contain three diploid CC genome species, *O. eichingeri*, *O. officinalis*, and *O. rhizomatis.* These species are endemic to Asia and Africa, have different genome sizes, and are known to possess agronomically-important traits for future rice breeding. With the aim of establishing a reference genome for the *Oryza* CC genome, we have been constructing a pseudomolecule of the *O. officinalis* genome, which has a moderate genome size of ~570 Mb and exits in the widest habitat of the CC genome species. To date we have generated and Illumina sequenced two paired end (210 and 350 bp), and three mate pair (2.4, 6, and 8 kb) libraries, and will incorporate additional libraries in order to produce long sequence scaffolds. After appropriate quality control and filtering steps a whole genome *de novo* assembly was conducted with MP scaffolding. Resultant scaffolds were then aligned to AGI's *O. officinalis* physical map using a BAC/BAC-end sequence-supported Super Scaffolding pipeline. Currently our assembly covers ~438 Mb of the *O. officinalis* genome (including ~40 Mb of Ns) with about 56,000 scaffolds supported by the AGI BAC-end sequences.

Keyword : wild rice, *Oryza officinalis*, genome sequencing NGS, De novo assembly

PA-02

Identifying the functional elements in the rice genome by comparisons of multiple *Oryza* genomes

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Previous comparative genomic analysis in several orthologous regions indicated a better annotation and potentiality for finding new functional elements in the rice genome. The emerging of complete genomic sequences of several *Oryza* (or grass) species has brought about fascinating opportunities to decipher the rice genome through whole genome comparative analysis. The platform for systematic genome interpretation based on evolutionary signatures was well established in yeast, fly and mammals. However, little has been done in plants. Here we show the preliminary results performed on the short arms of chromosome 3 among *Oryza* species based on multiple genomic alignments. Our first step is to revisit the protein-coding genes in the rice genome, aiming at giving a more accurate gene catalog and finding new protein-coding sequences.

Keyword : *Oryza* , whole genome comparative analysis, protein-coding genes, functional elements

PA-03

Comparative analysis reveals low synteny and gene escape in the rice centromeric regions

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Centromere is a functional chromosomal site that enables the accurate segregation of chromosomes during mitosis and meiosis. Rice has been proposed as a model for studying centromere structure and evolution. Upon BAC sequencing and assembly of centromeric regions in *O. brachyantha* CEN3 and CEN4, we compared the orthologous centromeric regions in *O. sativa*, *O. brachyantha*, *S. bicolor* and *B. distachyon* to reveal their evolutionary patterns. By analyzing the approximately 600-kb flanking region of the centromere satellite repeats in rice CEN4 and the orthologous regions from other species, two genes were found to move out, three genes forming a segmental duplication in the chromosome 4 long arm in *O. sativa*, and a conserved gene lost in *O. brachyantha*. Only three genes were found to be collinear among these four species. In CEN3, in the approximately 1000-kb region flanking the satellite repeats in rice, at least five genes move to other position in the genome, and only four orthologous genes retain their collinear positions. Many of the escaped genes have multiple exons and are potentially functional. Gene escape from centromeres in the form of individual gene or segmental duplication, may be associated with expansion of the centromere satellite repeats, transposon insertions and epigenetic modifications.

Keyword : centromere, synteny, gene escape

PA-04

Phylogenetic Inference from whole-genome sequence of Taiwan rice cultivars and landraces

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The artificial mating, natural introgression and selection gave different domestication force to, and became elite landraces. The segmental recombination were happened at the same time genome-wide. The target genes and their nearby regions inherited from one parent, but they might be broken during one meiosis later on. In this study, we would like to classify preserved and domesticated regions using NGS data. There are many upland rice landraces collected from different aboriginal villages in Taiwan, and they display divergence on many agronomy traits. These rice resources could be used as germplasm for rice improvement. Next-generation sequencing data of eleven accessions, containing 7 modern cultivars and 4 landraces of Taiwan, were compared with Nipponbare genomic sequence to reveal the variations during domestication and breeding process. The phylogenies were constructed with randomly selected regions over the entire genome. The distribution of polymorphism was examined to screen loci differential between cultivars and landraces which implied the key loci of domestication and breeding. The consensus tree of phylogenetic trees from all regions thus may provide an inference on the evolution history of cultivated rice in Taiwan.

Keyword : NGS, phylogenetics, landrace, domestication, variation

PA-05

Detection of reproductive barriers between Aus and *indica* rice.

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Study of reproductive barriers is important both for breeding and for understanding the genetics of speciation. *Oryza sativa* has two major subspecies, *japonica* and *indica.* Aus had been considered to be an ecotype of *indica,* however, recent molecular analysis showed Aus recently differentiated and formed a group genetically distinct from other *indica.* Many reproductive barriers between *japonica* and *indica* have been detected and some are cloned, however no reproductive barriers between Aus and *indica* have been reported. To detect reproductive barriers between the Aus variety Kasalath and the *indica* variety 93-11, we developed PCR co-dominant markers covering the whole genome using insertion/deletion between *japonica* Nipponbare and 93-11. About a quarter of investigated insertion/deletion polymorphisms between Nipponbare and 93-11 also showed polymorphisms between Kasalath and 93-11, and yielded 122 PCR markers. Genetic linkage maps were constructed using 5 different populations from crosses between Kasalath (K) and 93-11 (Q), two F_2 populations from reciprocal hybrids (K/Q F_2; 286 plants, Q/K F_2; 190 plants), and three backcross populations (K/Q//K; 140 plants, K//K/Q; 146 plants, K/Q//Q; 87 plants). Marker allele frequencies of the linkage maps were analyzed to detect reproductive barriers. The number of detected reproductive barriers between Kasalath and 93-11 was fewer than between Nipponbare and Kasalath or between Nipponbare and 93-11. A prominent peak of distorted allele frequencies from Mendelian expectation was observed near the long arm end of chromosome 6 in both F_2populations and K//K/Q. This distorted segregation could be caused by a male gametophyte barrier between Kasalath and 93-11.

Keyword : Aus, *indica*, reproductive barrier, distorted segregation

PA-06

Fine mapping of a QTL for the number of spikelets per panicle by using near-isogenic lines derived from an interspecific cross between *Oryza sativa* and *O. minuta*

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We constructed a high-resolution physical map for the *qSPP7* QTL for spikelets per panicle on rice chromosome 7 across a 28.6-kb region containing 4 predicted genes. Using a series of BC_7F_4 nearly isogenic lines (NILs) derived from a cross between the Korean *japonica* cultivar 'Hwaseongbyeo' and *Oryza minuta* (IRGC Acc. No. 101144), 3 QTLs for the number of spikelets per panicle (SPP), grains per panicle, and primary branches were identified in the cluster ($P \leq 0.01$). All 3 QTLs were additive, and alleles from the *O. minuta* parent were beneficial in the 'Hwaseongbyeo' background. *qSPP7* was mapped to a 28.6-kb region between the 2 simple sequence repeat (SSR) markers RM4952 and RM21605. The additive effect of the *O. minuta* allele at *qSPP7* was 23 SPP, and 43.6% of the phenotypic variance was explained by the segregation of the SSR marker RM4952. Colocalization of the 3 QTLs suggested that this locus was associated with panicle structure and had pleiotropic effects. The NIL populations and molecular markers are useful for cloning *qspp7*.

Keyword : Keywords: rice, spikelet per panicle, QTL, near isogenic line, fine mapping

PA-07

Mapping quantitative trait loci for spikelets per panicle using NILs carrying wild rice (*Oryza rufipogon*) chromosome segments in cultivar background

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In a previous study, QTL for spikelets per panicle, *qspp1* was detected on chromosome 1 using 96 BC_3F_8 lines derived from a cross between 'Hwaseongbyeo' as a recurrent parent and '*O. rufipogon*' as a donor parent. At this locus, the *O. rufipogon* allele increased SPP. Among the 96 introgression lines, three ILs with the *O. rufipogon qspp1* locus showed higher number of spikelets per panicle (SPP) than the recurrent parent. One line, CR572 was selected and crossed to 'Hwaseongbyeo' for fine-mapping *qspp1*. A total of 494 $F_{2:3}$ lines were evaluated for spikelets per panicle and agronomic traits in the field. QTL analysis in 494 $F_{2:3}$ lines indicated that the QTLs for spikelets per panicle was located in the interval RM495 – RM283. These results suggested that the *qspp1* locus could be useful in improving yield potential of japonica cultivar.

Keyword : Rice, QTL, spikelets per panicle

PA-08

Centromere evolution accompanied by inversions and transpositions in the *Oryza* species

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We completely sequenced the genome of *Oryza brachyantha,* which belongs to the FF genome type in the genus *Oryza*. The divergence time between *O. brachyantha* and rice (*Oryza sativa)* is approximately 15 million years. By comparing the sequences in peri-centromeric regions for all the 12 chromosomes between these two species, we identified inversions in 5 chromosome centromeric regions (chr3, chr5, chr9, chr8, and chr10). The inversion sizes range from a few hundred kilo-bases to 1 megabases. Four centromeric locations (chr6, chr8, chr9, and chr12), defined by the locations of the largest CentoF/CentO arrays, were found to be shifted. We further verified these events by using the BAC-based physical maps (http://www.omap.org). We sequenced (using 454 technology) 43 BAC clones in peri-centromeric regions for chromosomes 5, 6, 9, 10, and 12. The sequence assembly fully confirmed the above findings. Previous study on rice Cen8 revealed an inversion and transposition in the centromeric region. Sequence analysis of Cen9 revealed similar results. Our findings strengthened the strategy to study centromere evolution genus-wide using a single centromere (cen8) as a model. We also provided the potential to clarify centromere evolution by inter-chromosomal comparisons.

Keyword : *Oryza*; centromere evolution; inversion; transposition; inter-chromosomal comparisons;

PA-09

Sequencing analysis of domestication related genes of rice landraces collected from Taiwan aboriginal villages

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Taiwan aboriginal peoples are thought to be the origin of Austronesians. Currently, there are 14 tribes of aboriginal people in Taiwan and they use different languages. The major crops in the aboriginal villages are rice (*Oryza sativa*) and foxtail millet (*Setaria italica*). Sixty rice landraces collected from Taiwan aboriginal villages about 100 years ago were used in this study. These rice landraces show large variation in phenotype, included grain size, plant size and architecture, aboveground biomass, heading habitat and drought tolerance etc. Several rice domestication-related genes responsible for heading date, grain size, seed color and dormancy have been cloned recently. In this study, DNA sequences and Targeting Induced Local Lesions in Genomes (TILLING) analysis illustrate many DNA sequence variations including SNP, deletion and insertion in these domestication- related genes. In addition, 52 SSR markers distributed on 12 chromosomes were used to evaluate the genetic diversity and phylogenetic relationship of these landraces. Several loci showed high PIC value and thus indicates that there are significant genetic variations among them.

Keyword : landrace domestication-related genes

PA-10

Introgression pattern on rice domestication history inferred from phylogenomic analysis

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This study aims to clarify the pattern and extent of introgressions between varietal groups of Asian rice, *Oryza sativa*, which led to generation of the group specific genetic background. Previous studies have indicated that two major groups of Asian rice, *japonica* and *indica*, had different wild progenitor populations of *O. rufipogon* and underwent hybridizations that resulted in introgressions of key domestication genes. We conducted genome-wide phylogenetic analysis using recently published genome re-sequencing data of 40 domesticated and 10 wild rice strains as well as two newly sequenced genomes of India and Malaysia. Our NGS read mapping could cover 38% of the Nipponbare reference genome with uniquely placed reads of all 52 accessions. As a result, more than 3 million SNPs were detected. We conducted phylogenetic analysis with 10-kb window for the entire genome, and found 6% of the genomic regions have experienced introgression between *temperate japonica* and *indica*. Although the direction of introgression between *temperate japonica* and *indica* was mainly unidirectional from *japonica* to *indica*, it was also indicated that other *japonica* varietal groups, *tropical* and *aromatic japonica*, have a significant amount of introgressed regions derived from *indica*. In addition, the *aus* group was found to have greater amount of *japonica*-derived genomic regions than the pure *indica* group. These results suggests that introgression between cultivars played a major role to generate the genetic makeup of the todays rice varietal groups.

Keyword : introgression, NGS, phylogenomics, domestication

PA-11

Indica-japonica subspecies-specific InDel loci: A novel approach for understanding evolutionary relationships in the genus *Oryza*

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Subspecies-specific (SS) markers are linked to highly conserved genomic regions specific to the *indica* and *japonica* subspecies of *Oryza sativa*. The evolutionary relationship among *Oryza* species was investigated by the amplification of genomic DNA from a total of 290 accessions, including 61 Asian cultivated rice (*O. sativa*) cultivars, 27 African cultivated rice (*O. glaberrima*) accessions, and 202 accessions of wild *Orzya* species, using 67selected *indica-japonica* insertion-deletion (InDel) SS-STS primers. As expected, the average SS allele frequency of AA species, BB~EE, and FF~HHKK showed an increased proportion of non-*O.sativa* and null alleles in the more distantly related wild species. Most of the wild species, except the more distant EE, GG, HHJJ, and HHKK genome accessions, consisted of relatively more *indica* alleles of SS markers. To validate the genetic relationship study using the gel based SS-STS InDel markers, PCR products of nine markers were sequenced across 24 to 33 accessions. Sequencing results revealed that *Oryza* species share *indica* or *japonica*-like conserved InDel regions even across the different genomes. This study demonstrated how SS-STS InDel polymorphisms can trace back alleles associated with *indica* or *japonica* over evolutionary time and through domestication. The presence of some *japonica* alleles beyond the AA genome at some SS InDel loci also supports the possibility that the advent of *japonica* specific alleles occurred where *Oryza* genus appeared. This work was supported by a grant from the Next-Generation BioGreen 21 Program (Plant Molecular Breeding Center, No. PJ009076), Rural Development Administration, Republic of Korea.

Keyword : *Indica*/*japonica* specific, wild relatives, *Oryza*

PB-01

Genome-wide analysis of differentially expressed genes between *japonica* and *indica* rice

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Changes in gene expression are thought to underlie many of the phenotypic differences between species. Here we report characteristic features of genes expressed differentially between rice subspecies, including their evolution and expression patterns. For large-scale analyses of gene expression, 321 datasets of the Affymetrix Rice Genome Array, containing 121 arrays of different *japonica* tissues and 200 arrays of different tissues of three *indica* varieties were obtained from the Gene Expression Omnibus (GEO) and data from 6 tissues were analyzed to detect *japonica-indica* differentially expressed (*ji*DE) genes. Of all 43,998 genes on the arrays, 21,238 (48%) were highly expressed in *japonica* at least one of 6 tissues and 14,924 genes of 43,998 total genes had similar expression (SE) between *japonica* and *indica*. Although the number of highly expressed genes was similar across tissues of *japonica* and three *indica* varieties (about 13,000), the number of *ji*DE genes varied (585~1,184). In total 5,384 *ji*DE genes were detected at least one of 6 tissues and we found that *ji*DE genes had two types of expression patterns, being either constitutive or tissue dependent expression. Finally, to assess the selection pressures acting on *ji*DE, the ratio of nonsynonymous and synonymous substitution rates of *ji*DE genes were compared with ones of SE genes. Rapidly evolved genes were enriched in constitutive *ji*DE genes but not in tissue dependent *ji*DE. Studying functional descriptions of *jiDE* by gene ontology enrichment analysis, it was observed that "apoptosis" and "defense response" assigned genes were enriched in constitutive type *ji*DE (Fisher's $p < 10^{-5}$).

Keyword : gene expression, microarray, gene evolution, *japonica-indica* differentiation

PB-02

Developmental and physiological functions of rice root revealed by comprehensive transcriptome profiling

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The root system performs many essential functions including water and nutrient uptake and anchorage to the soil for plant growth. Elucidating the molecular mechanism of root development and functions is therefore necessary for improving plant productivity, particularly for crop plants, including rice (*Oryza sativa*). Here, in order to gain a better understanding of the formation and function of the root system, we performed genome-wide transcriptome analysis of the rice root via a combined laser micro dissection and microarray approach. We analyzed a total of 38 microarray data corresponding to eight developmental stages along the longitudinal axis and three radial tissue types at two different developmental stages, and identified 22,297 genes corresponding to 17,010 loci with sufficient signal intensity. We clarified gene networks associated with root cap function and lateral root formation, and further revealed antagonistic and synergistic interactions of phytohormones, based on the expression pattern of phytohormone-related genes. Furthermore, expression profiling of transporter genes defined not only the major sites for uptake and transport of water and nutrients, but also distinct signatures of the radial transport system from the rhizosphere to the xylem vessel for each nutrient. These results suggest that our comprehensive gene expression profile will be very useful not only for elucidating gene regulatory networks of the root system but also for exploring valuable genes in forward- and/or reverse-genetics approaches, which could lead to novel strategies for crop improvement.

Keyword : rice, root, laser microdissection, microarray, database

PB-03

Genome information database system for innovation of crop and livestock production - the current progress of the development of a common infrastructure for genome analysis

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In agricultural genetic research, with the progress in genome sequencing, the identification of important genes and the development of DNA markers have been proceeding at a rapid pace. The next generation sequencing (NGS) technologies produce large amounts of data, and applying these technologies is leading to increasing demand for computing power and storage capacity. Here, we present the current progress of the development of a database system devoted to agriculture genome information. We construct a database to provide comprehensive and accessible genome information, and we develop a system that would be helping researchers effectively analyze NGS data, locate new genes and develop DNA markers associated with important traits. For this purpose, two disks that have a total storage capacity of 2 petabytes and 100 node cluster were created. The cluster nodes are connected to the disks by 10 GBit network. The aim of this system is the creation of the database for rice genome information including cultivars and wild relatives, with the plan to extend it with the genome information of other crops. In order to provide the comprehensive information related to the genes of interest with expanded information about the gene annotations, expressions, etc., a database browser will be integrated with the system interface. We have already created two contents "Sequencing Data Management" and "Research Data Management" for managing large quantities of NGS data. To analyze the sequence data by BLAST and FASTA programs, we have developed a framework for using these programs from our system. We have added in this framework alignment and mapping tools like BWA and Bowtie for analysis of NGS data. Moreover, we integrated graphics processing units (GPUs) into our system. Therefore, we add in our analysis service the developed applications and published software packages that require GPU environment. In this database system we can collect and manage a broad range of data from sequencing reads to results of data analysis, and all these data can be easily used by registered users with a variety of tools provided in the system. In addition, we have added the links to the existing databases and services provided by our institute, and they can be accessed from our system. We hope that our system will assist researchers to identify agronomically important genes, and will contribute to improving the efficiency of breeding.

Keyword : database, next generation sequencing

PB-04

RiceXPro and RiceFREND: Cyclopedic databases of rice gene expression profiling

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Gene expression profiling is an effective approach to reveal the functions of all genes in rice. We have developed two databases, RiceXPro (http://ricexpro.dna.affrc.go.jp/) and RiceFREND (http://ricefrend.dna.affrc.go.jp/), based on the gene expression profiles of the rice plant in the field and various experimental conditions. RiceXPro is a database designed as a repository of gene expression profiles derived from microarray analysis. The current version contains 753 microarray data classified into 26 datasets and categorized as 'field/development', 'plant hormone' and 'cell- and tissue-type'. In order to characterize the gene expression of each gene, 4 types of interfaces are provided including 'keyword search', 'chromosome/region search' and 'analysis tools' for retrieving the data statistically, and 'EXP_BLAST' to explore the gene expression profile with similarity to a query sequence. The expression data are shown in table, graph, or heatmap formats. RiceFREND is a database of gene coexpression networks in rice. The gene coexpression analysis is performed based on 24 datasets representing 815 microarray data. Coexpression analysis is performed via 'single guide gene' and 'multiple guide genes' searches in which a pre-selected gene or set of genes is used as 'guide gene' to retrieve coexpressed genes. These genes can be viewed in HyperTree, Cytoscape Web or Graphviz formats in addition to tabular format. Additional tools such as 'GO enrichment test' and 'cis-element analysis' are provided for further assistance in figuring out gene function. These databases could be used as powerful tools for functional genomics and may contribute in understanding many complex agronomic traits in rice.

Keyword : database microarray expression coexpression-network gene-function

PB-05

IRRI GALAXY: Enabling bioinformatics for rice scientists

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The continuous decline in DNA sequencing costs has made genome resequencing increasingly the method of choice for marker-assisted breeding and population characterization of important crops. At the International Rice Research Institute (IRRI), two research program themes have adopted Next-Generation Sequencing (NGS) technologies for characterizing diversity of the rice gene bank, creating novel gene pools for utilization in breeding, and discovering novel genes/alleles underlying QTLs that confer valuable traits. The data throughput of NGS is overwhelming for breeders and molecular biologists, and specialized software tools usually having command-line interfaces are required to handle the raw/analyzed data coming from NGS. Commercial NGS analysis software with slick, easy to use graphical user interfaces (GUI) usually costs thousands of dollars. In the human genomics community, the development of an Open Source web application, GALAXY (http://galaxyproject.org/), has provided GUIs and workflow management of common and repetitive bioinformatics tasks for the analysis of NGS datasets. We adopted GALAXY as bioinformatics resource, and added to the GALAXY tools our in-house developed software specific to the needs of the research programs at IRRI : custom SNP allele calling for Beadstudio platforms, automatic reformatting of SNP datasets for use in several genetic mapping/diversity analyses applications, and management of huge data matrices. The IRRI GALAXY resource is freely available to the rice research community, and other GALAXY resources can obtain the tools developed in IRRI GALAXY via the GALAXY TOOLSHED site, the apps "store" for GALAXY.

Keyword : Galaxy Bioinformatics Workbench SNP

PB-06

The rice South Green hub

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The South Green Bioinformatics Platform, http://southgreen.cirad.fr/, is dedicated to the genetics and genomics of tropical and Mediterranean plants. We focus here on the resources and tools useful for rice functional genomics. OryGenesDB is a database developed for rice reverse genetics. OryGenesDB contains FSTs (flanking sequence tags) of various mutagens and functional genomics data collected from both international insertion collections and the literature. Oryza Tag Line is the phenotype equivalent of OryGenesDB. It lists all the morphological and physiological data gathered on DNA-T insertion lines. Coupling these two bases will enable the rapid identification of the effect of a mutation in a given gene, by looking into the morphological and physiological characteristics of the corresponding plants. The most common data stored in TropGeneDB are molecular markers, QTLs, genetic and physical maps, genetic diversity, phenotypic diversity studies, and information on genetic resources. Indeed, the TropGeneDB rice module stores unique genetic and phenotypic data on European collections of rice. GreenPhylDB, has been designed for comparative and functional genomics in plants. The database contains a catalogue of gene families based on complete genomes, covering a broad taxonomy of green plants, including rice and other cereals. This ortholog prediction is particularly useful for functional genomics and candidate gene identification of genes affecting agronomic traits of interests. All these information systems are closely linked. We also developed Galaxy pipelines and many other systems: community system for structural and functional annotation, analysis of RNA-Seq, SNP detection, etc.

Keyword : Bioinformatic, Database, Mutagenesis, Comparative Genomics

PC-01

Improving rice with maize genomic resource, a promising approach

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BIBAC system has been developed for functional genomics studies through *Agrobacterium*-mediated transformation of large genomic DNA fragments into plants. We previously modified BIBAC and BAC vectors (Shi *et al*., 2011, Plant Methods 7:33) that can largely facilitate BAC/BIBAC library construction and exchange of intact large insert DNA fragment between the BIBAC vector and the BAC vector. A maize B73 BIBAC library was constructed using our modified BIBAC vector. Stability test showed that BIBAC clones with different insert sizes from 40 kb to >160 kb were all stable in *Agrobacterium* EHA105 after cultured for at least 96 h (4 days), the time length that are needed to complete co-inoculation with rice callus in rice transformation. We propose to construct transgenic rice lines with tiling maize B73 BIBACs. To date, more than 1000 B73 BIBAC clones were end-sequenced and more than 2000 BIBAC clones were used for rice transformation. We have demonstrated that an 164-kb maize B73 genomic DNA fragment with 88.1% repeat sequences and containing 5 filtered gene models was completely integrated into rice genome and the maize gene models were expressed in the transgenic rice plants. Some BIBAC transgenic rice plants with changed phenotypes possibly from gain of function of maize genes or loss of function of rice genes have been obtained and are being further analyzed. More information can be obtained through our website http://GResource.hzau.edu.cn.

Keyword : BAC, BIBAC, Rice, Maize

PC-02

Rice *MPR25* encodes a pentatricopeptide repeat protein and is not only essential for RNA editing of *nad5* transcripts in mitochondria but for chloroplast function.

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Pentatricopeptide repeat (PPR) proteins are defined by the tandem array of PPR motif each consisting of 35 amino acids, and they form a huge gene family in land plants. PPR proteins are involved in the modification of organelle transcripts. In this study, we investigated the molecular function in rice of mitochondrial PPR-encoding *MITOCHONDRIAL PPR 25* (*MPR25*), which belongs to the E subgroup of the PPR family. The *Tos17*-knockout mutant exhibited growth retardation and pale-green leaves with reduced chlorophyll content during the early stages of plant development. Photosynthetic rate in the *mpr25* mutant was significantly decreased, especially under strong light conditions, although the respiration rate did not differ from that of wild-type plants. *MPR25* was preferentially expressed in leaves. FLAG-tagged MPR25 accumulated in mitochondria but not in chloroplasts. Direct sequencing revealed that the *mpr25* mutant fails to edit a C-U RNA editing site at the 1580th nucleotide of *nad5,* which encodes a subunit of complex I (NADH dehydrogenase) of the respiratory chain in mitochondria. RNA editing of this site was considered to be responsible for a change in amino acid from serine to leucine. Recombinant MPR25 directly interacted with the proximal region of the editing site of *nad5* transcripts. NADH dehydrogenase activity of complex I, however, was not affected in the mutant. By contrast, genes encoding alternative NADH dehydrogenase and alternative oxidase were up-regulated. The *mpr25* mutant could therefore provide new information on the coordinated interaction between mitochondria and chloroplasts (Toda et al. 2012 Plant J. Online).

Keyword : mitochondria, *nad5*, *Oryza sativa* L., pentatricopeptide repeat, RNA editing

PC-03

Genetic and phenotypic characterization of giant embryo mutants in rice.

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As a vital human nutrient and industrial material of economic value, rice embryo has been a prime target of breeding efforts through which new varieties of larger embryo had been sought after. Moreover, with recent reports that the contents of several health dietary supplements present in rice, such as gamma aminobutyric acid (GABA), tocopherol and other vitamins, are positively correlated with the embryo size, the field of embryo research is receiving ever-increasing attention. Through chemical mutagenesis of Hwacheongbyeo (a temperate *japonica* cultivar) with *N-methyl-N-nitrosourea* (MNU), we obtained three mutants,*ge*, *ge-m*, and *ge-s*, each of which represents distinct group of larger embryo size than the wild type. Genetic crosses and direct sequencing of these mutants in the region of a known embryo size-controlling gene, *GE* (Giant Embryo), revealed that *ge* and *ge-s* were allelic to the *GE* with two independent point mutations in the coding region. On the other hand, *ge-m* mutant, the embryo of which featured intermediate size in between those of *wild type* and *ge*, turned out to be non-allelic to the *GE* locus, suggesting it is likely a novel gene, which influences rice embryo development through a different mechanism than *GE* gene. Fine mapping of *ge-m* gene is currently in progress.
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Keyword : Giant Embryo, Embryo development, GABA, *ge-m, ge-s*

PC-04

OsRAD51D, one of the rice *RAD51* paralogs, participates in reproductive organ formation and seed development in rice (*Oryza sativa* L.)

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RAD51 is one of the key components in homologous recombination (HR) in eukaryotes. In addition to RAD51, mammalian cells contain five RAD51-like proteins (RAD51B, RAD51C, RAD51D, XRCC2, and XRCC3), which play a role as mediators of HR. Here, we characterized *OsRAD51D* that encodes a DNA repair- and HR-associated protein in rice. T-DNA knockout *osrad51d* mutant and *Ubi:OsRAD51D-RNAi* knockdown lines were constructed and their phenotypes were characterized. Terminal restriction fragment analysis revealed that telomeres in mutants were longer than those of wild type plant. Homozygous G3 *osrad51d* plants showed normal vegetative growth. However, the mutant displayed severely impaired reproductive organs and anther dehiscence, resulting in sterile flowers. *osrad51d* exhibited abnormal pollen development, which led to disrupted pollen viability. Heterozygous mutant and *RNAi* plants also showed lower seed filling rates and their mature seeds displayed opaque phenotype composed of abnormal starch granules and multi-layered aleurone layers. Transcript of seed development related genes were reduced in *osrad51d* mutant progeny. The promoter region of *OsRAD51D* contains GCN4 and skn-1 motifs, both of which are required for endosperm development. Gel retardation assay showed that RISBZ, an important transcription factor in seed development, binds to OsRAD51D promoter *in vitro*. Cytogenetic analysis represented meiotic defects in *osrad51d* pollen mother cell. These results suggest that OsRAD51D participates in reproductive organ formation and seed development in rice. This work was supported by grants from the Next Generation BioGreen 21 Program (PJ008152) and the Korea Polar Research Institute (PE11020) to WTK. LC was recipient of BK21 scholarship.

Keyword : *RAD51D*, homologous recombination (HR), telomere, reproductive organ formation

PC-05

Ectopic expression of *OsMADS45* activates the upstream genes *Hd3a* and *RFT1* at an early development stage causing early flowering in rice

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The rice gene, *OsMADS45*, which belongs to the MADS-box E class gene, participates in the regulation of floral development. Previous studies have revealed that ectopic expression of *OsMADS45* induces early flowering. However, the regulation mechanism of *OsMADS45* overexpression remains unknown. We introduce an *OsMADS45* overexpression construct *Ubi:OsMADS45* into TNG67 plants (an *Hd1* (*Heading date 1*) and *Ehd1* (*Early heading date 1*) defective rice cultivar grown in Taiwan), and analyzed the expression patterns of various floral regulators to understand the regulation pathways affected by *OsMADS45* expression. The transgenic rice exhibit a heading date approximately 40 days earlier than that observed in TNG67 plants, and transgenic rice display small plant size and low grain yield. *OsMADS45* overexpression did not alter the oscillating rhythm of the examined floral regulatory genes but advanced (by approximately 20 days) the up-regulate of two florigens, *Hd3a* (*Heading Date 3a*) and *RFT1* (*RICE FLOWERING LOCUS T1*) and suppressed the expression of *Hd1* at the juvenile stage. The expression levels of *OsMADS14* and *OsMADS18*, which are two well-known reproductive phase transition markers, were also increased at early developmental stages and are believed to be the major regulators responsible for early flowering in *OsMADS45*-overexpressing transgenic rice. *OsMADS45* overexpression did not influence other floral regulator genes upstream of *Hd1* and *Ehd1*, such as *OsGI (OsGIGANTEA)*, *Ehd2/Osld1/RID1* and *OsMADS50*. These results indicate that in transgenic rice, *OsMADS45* overexpressing ectopically activates the upstream genes *Hd3a* and *RFT1* at early development stage and up-regulates the expression of *OsMADS14* and *OsMADS18*, which induces early flowering.

Keyword : Floral regulatory genes, *OsMADS45*, *RFT1*, *Hd3a, Hd1*

PC-06

FCA/ABAP1 is an important enhancer in ABA signaling in rice seed germination

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ABAP1, a truncated form of FCA, was first reported as an abscisic acid (ABA) binding protein in barley aleurone cells; yet literatures regarding the function of this protein in barley and in Arabidopsis have been controversial. Following a multidisciplinary approach combining molecular genetics, biochemistry, cell biology, and physiology, we have obtained strong evidence to suggest that FCA/ABAP1 is an important enhancer in ABA signaling in cereal grains. Over-expression of FCA/ABAP1 enhances, while its RNAi suppresses, rice seed germination and the ABA up-regulation of LEA protein synthesis. Epistasis studies indicate that FCA/ABAP1 works upstream from VP1/ABI5, which are important signaling molecules/transcription factors for ABA up-regulation of LEA protein expression. The FCA/ABAP1-GFP fusion protein is initially localized in the cytoplasm with a punctate pattern but then gradually translocated into nucleus. This cytosol to nucleus translocation of ABAP1 is further enhanced by ABA treatment. However, a major suppressor of ABA action, abi1, a dominant mutant of protein phosphase 2C, inhibits this cytosol/nucleus translocation of FCA/ABAP1. In planta two-hybrid studies reveal that FCA/ABAP1 interacts with VP1. Mutation of the highly conserved WW domain in FCA/ABAP1 suppresses nuclear translocation, disrupts FCA/ABAP1-VP1 interaction, and also suppresses ABA signaling. Although FCA/ABAP1 does not appear to bind ABA as demonstrated by the surface plasmon resonance (Biacore) technique, our results suggest that FCA/ABAP1 plays a pivotal role in ABA signaling by transmitting ABA signaling from cytosol to nucleus where this protein interacts with the transcription factor complex of VP1/ABI5 that are required for ABA up-regulation of gene expression.

Keyword : Rice seed germination, ABA signaling

PC-07

A large-scale of functional verification of domestication-related genes in rice

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Rice is not an important food crop, but also a good model to study crop domestication. Some key traits have changed during our ancestors domesticated wild rice to cultivar, including shattering, plant architecture, the colour of pericarp and hull, awnness, seed dormancy and so on. Some genes related these traits have been cloned, such as *Sh4* and *Prog1*. However, almost these genes were cloned through map-based cloning, which costed much time and work. Here, we identified 14 significant selective sweeps in all *japonica* genome and *indica* genome by analyzing our re-sequencing data of 50 accessions of rice, which is consistency with the domestication-related QTLs detected previously, including shattering, plant architecture, awnness, seed dormancy, stigma exertion and so on. Meanwhile, we identified 23 fixed-sites genes in these 14 selective sweeps of the cultivar genome. Then we transformed the wild rice alleles of 23 fixed-sites genes into a kind of short growth length cultivar, kitaake to verify whether the cultivar will occur the specific traits of wild rice. We want to clone some domestication-related genes in rice through this method which will save time and work. Now the work is ongoing.

Keyword : rice, domestication-related genes, reverse genetics

PC-08

Effect of light intensity on nitrogen uptake and assimilation in rice seedlings

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Nitrogen is one of plant essential nutrients. Nitrogen assimilation has been marked effects on crop development and production. The objective of this research is to reveal the influence of different photosynthetic photon flux density (PPFD; 50, 250 and 450 μmol m^{-2} s^{-1}) on nitrogen uptake and assimilation in rice seedlings. The result showed that rice seedling grown at PPFD of 450 μmol $m^{-2}s^{-1}$ had better growth vigor compared with that of seedlings grown under low-PPFD conditions. High light intensities promote uptake of ammonium-nitrogen source (but not nitrate-nitrogen source) from hydroponic solution. Expressions of *ammonium transporter* (*OsAMT1.1*) and *nitrate transporter* (*OsNRT1.1*) genes were determined in different PPFD conditions. In root tissues, *NRT1.1*mRNA levels were not obviously different in various light intensity conditions; however, *AMT1.1* expressions were significantly enhanced by high-PPFD (i.e. 450 μmol m^{-2} s^{-1}). On the other hand, both *AMT1.1* and *NRT1.1* expressions in shoot tissues were repressed at high-PPFD conditions. Moreover, nitrate reductase (NR) activities of seedlings grown at 450 μmol m^{-2} s^{-1} were higher than that was at 50 μmol m^{-2} s^{-1} in root tissues, and glutamine synthetase (GS) activities were slightly enhanced in both roots and shoots if PPFD were increased. Our data showed NR and GS activities were increased by exogenous sucrose in seedlings grown at low PPFD conditions. Thus, a hypothesis was proposed that activities of nitrogen assimilation-related enzymes regulated by light intensity could be mediated photosynthetic product such sucrose.

Keyword : Light intensity, Nitrogen assimilation, Nitrogen uptake

PC-09

The Rice FUnctional GEnomics (REFUGE) platform: an assessment of 4 years in hosting research projects using rice as a model system for functional analysis

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Since its establishment in Montpellier (France) end of 2008, the International hosting platform on RicE FUnctional GEnomics (REFUGE), http://www.refuge-platform.org/, has been assisting scientists and students from academic institutions of the North and the South, in the functional analysis of their favourite gene(s) using rice as a model system. Twenty-six research projects have been hosted gathering 15 different nationalities on the platform. Twenty (77%) projects are conducted by students from emerging or developing countries for an average hosting period of 10 weeks. Most of the projects aimed at deciphering the function of genes involved in developmental processes and/or tolerance to abiotic stresses. The platform staff provided the trainees with assistance in bioanalysis, vector construction, transformation, and molecular characterization of transformants as well as access to the cell imaging and stress phenotyping facilities. Seven PhD theses reporting experiments conducted on the REFUGE platform have already been defended and 15 are in preparation. Nine publications have appeared in international journals and a large number are being prepared. This assessment will be presented to funding agencies in 2013, for hopefully preparing a new phase of hosting that would resume in 2014.

Keyword : Platform, Rice, Functional genomics

PC-10

Artificial loci for evaluating homologous recombination efficiency in rice

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Enhancement of foreign DNA integration through homologous recombination (HR) in the nuclear genome of higher plants can be attempted through a manipulation of the plant recombination protein machinery and/or high frequency creation of double strand breaks at a desired target site. Up-regulation of proteins specific to the HR and down regulation of proteins specific to the Non Homologous End Joining (NHEJ) in one hand, and expression of nucleases possessing a DNA sequence recognition domain, on the other hand, have been used to reach these respective goals. Given the very low natural frequency of HR mediated DNA repair in somatic cells of higher plants it is necessary to implement versatile assays allowing an easy and early monitoring of these events. In that aim, we have established in rice two assays each based on an artificial T-DNA locus. The first system relies on the intermolecular recombination substrates successfully developed in *Arabidopsis* (Molinier et al 2004 The Plant Cell). Natural HR events lead to the reconstruction of a functional LUC or GUS gene that can only occur through intermolecular recombination. This system is particularly suited to assess the influence of mis regulation of recombination proteins on HR frequency. We have established a series of homozygous lines harbouring these substrates exhibiting a 1-6% frequency of LUC/GUS focus in seed embryo-derived callus lines upon histochemical assays. The second assay is based on the reconstruction of a functional *gfp* gene through introduction of a DNA repair substrate complementing a residing, artificial T-DNA locus at different locations in the genome. This assay proved to be functional in allowing the reconstruction of a functional *gfp* transgene at the expected 10^{-4} frequency in transformed callus lines. This assay is particularly suited to test the efficiency of cleavage by a nuclease at a target site in promoting HR-mediated repair.

Keyword : Homologous recombination (HR), DNA repair substrate, Artificial T-DNA, Double strand breaks

PC-11

Involvement of tonoplast-localized sucrose transporter, *OsSUT2*, in long-distance sucrose transport in rice

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Physiological functions of sucrose (Suc) transporters (SUTs) localized to the tonoplast in higher plants are poorly understood. We here report the isolation and characterization of a mutation in the rice (*Oryza sativa*) OsSUT2 gene. Expression of OsSUT2-green fluorescent protein in rice revealed that OsSUT2 localizes to the tonoplast. Analysis of the OsSUT2 promoter::beta-glucuronidase transgenic rice indicated that this gene is highly expressed in leaf mesophyll cells, emerging lateral roots, pedicels of fertilized spikelets, and cross cell layers of seed coats. Results of Suc transport assays in yeast were consistent with a H^+-Suc symport mechanism, suggesting that OsSUT2 functions in Suc uptake from the vacuole. The *ossut2* mutant exhibited a growth retardation phenotype with a significant reduction in tiller number, plant height, 1,000-grain weight, and root dry weight compared with the controls, the wild type, and complemented transgenic lines. Analysis of primary carbon metabolites revealed that *ossut2* accumulated more Suc, glucose, and fructose in the leaves than the controls. Further sugar export analysis of detached leaves indicated that *ossut2* had a significantly decreased sugar export ability compared with the controls. These results suggest that OsSUT2 is involved in Suc transport across the tonoplast from the vacuole lumen to the cytosol in rice, playing an essential role in sugar export from the source leaves to sink organs.

Keyword : rice, sucrose transporter, tonoplast, phloem loading, sucrose

PC-12

Characterization of rice monosaccharide transporters involving the vacuolar sugar transport

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In Arabidopsis, the compartmentation of sugars into vacuoles is known to be facilitated by sugar transporters such as tonoplast monosaccharide transporter (*AtTMT*) and vacuolar glucose transporter (*AtVGT*). However, vacuolar sugar transporters have not been studied in detail in other plant species so far. To characterize the rice (*Oryza sativa*) tonoplast monosaccharide transporters, we isolated two rice *TMTs*, *OsTMT1* and *OsTMT2*, further we analyzed their subcellular localization using green fluorescent protein (GFP) and expression patterns using reverse-transcription polymerase chain reaction (RT-PCR), performed histochemical b-glucuronidase (GUS) assay and in situ hybridization analysis, and assessed sugar transport ability using isolated vacuoles. As a result, expression of *OsTMT*–GFP fusion protein in rice and Arabidopsis clearly demonstrated that the OsTMTs localized at the tonoplast. Analyses of *OsTMT* promoter-GUS transgenic rice indicated that *OsTMT1* and *OsTMT2* are highly expressed in bundle sheath cells, and in vascular parenchyma and companion cells in leaves, respectively. Both genes were found to be preferentially expressed in the vascular tissues of roots, the palea/ lemma of spikelets, and in the main vascular tissues and nucellar projections on the dorsal side of the seed coats in consistent with RT-PCR result. Glucose uptake studies using vacuoles isolated from transgenic mutant Arabidopsis (tmt1-2-3) expressing OsTMT1demonstrated that *OsTMTs* are capable of transporting glucose into vacuoles. Based on expression analysis and functional characterization, our present findings suggest that the *OsTMTs* play a role in vacuolar glucose storage in rice.

Keyword : monosaccharide, rice (*Oryza sativa*), sugar transporter, tonoplast, vacuole

PC-13

Sucrose transporter expressions regulated by mechanical wounding in rice seedlings

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Plants often suffer from mechanical damage caused by herbivores attack, pathogen infection, strong wind or heavy rain. Sugars unloaded from phloem at wound sites provide the energy and carbon source for tissue healing. In addition, sugars may also function as signals to trigger some wounding responses. Sucrose transporters (SUTs) are key carriers to load sucrose into phloem in source tissues or unload sucrose from phloem in sink tissues. Rice *SUT* gene family is composed of five members, *OsSUT1* to *OsSUT5*. The results of gene expression analyses showed that both *OsSUT2* and *OsSUT4* mRNA levels were significantly increased in wound-treated leaf tissues; however, there was no obvious wound effect on *OsSUT1* expressions. Furthermore, *OsSUT2* and *OsSUT4* promoter activities were observed in wounded leaf tissues of *OsSUT2* promoter::*GUS* and *OsSUT4* promoter::*GUS* transgenic rice plants. Since the expression pattern of *cell wall invertase 1* gene in wounded leaves was correlated with that of *OsSUT2* and *OsSUT4*, it was considered both *OsSUT2* and *OsSUT4* play an important role to transport sucrose to wounded site. Following, sucrose unloaded from phloem was further hydrolyzed to hexose by cell wall invertase for supporting the tissue repairing. Moreover, since the wound-enhanced *OsSUT4* expressions were repressed by oxylipin biosynthesis inhibitor, aspirin, it was suggested that oxidized fatty acids or their derived metabolites might be involved in the mechanism of wound-regulated *OsSUT4* expressions. This hypothesis was supported by the up-regulated expressions of genes encoded allene oxide synthase (*OsAOS*, a key enzyme in charge of oxylipin biosynthesis) stimulated by wound-treatment in rice leaf tissues.

Keyword : mechanical wounding, sucrose transporter

PC-14

Arbuscular mycorrhizal symbiosis: Its effect on rice Pi uptake

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Phosphorus (Pi) is an essential macro element for rice production. However, P is not easily available to plants since it may be fixed in the soil. Phosphorus deficiency severely limits rice production and plants therefore evolved and utilize mechanisms to cope, such as entering into symbiotic association with the arbuscular mycorrhiza (AM) fungi. More than 80 percent of terrestrial plants, including rice, enter into symbiosis with the AM, a fungi that grows inter- and intracellular in the root cortex (Smith et al, 1997). This association enables plant to derive certain amount of nutrients, particularly Pi, via the mycorrhizal uptake pathway. In this study, the effect of AM symbiosis in the Pi uptake pathway of different rice varieties grown in soils derived from natural irrigated and upland plots was assessed. AM colonization in rice roots was quantified using rice AM marker genes. AM1 and AM3 showed early and continuous induction, whereas AM14 was activated at later stages indicating natural colonization of roots. The direct and mycorrhizal Pi acquisition pathways were distinguished by quantifying expression of different phosphate transporter genes, namely PT2 and PT6 specific to the direct uptake pathway, and PT11 and PT13 which are specific to AM colonized roots. The expression profiles revealed genotypic differences, as well as different degrees of colonization in the tested soils. However, no correlation was found between mycorrhizal colonization and overall plant vigor (i.e. plant height, tiller count, and grain yield).

Keyword : arbuscular mycorrhiza, symbiosis, Pi nutrition

PC-15

Engineering a two-celled C4 photosynthetic pathway in rice

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The ability to increase crop yield by 50% while also improving nitrogen and water use efficiency is just what is needed to kick start the next green revolution and feed a growing global population. It has been widely reported that this can be achieved through the installation of a C4 photosynthetic pathway into a C3 crop such as rice. While the evolution of C4 photosynthesis provides a biological precedence for this being possible a significant number of challenges lie ahead before this can be achieved. While the basic biochemistry of C4 photosynthesis has been understood for 50 years there are still many aspects that remain unexplained. Furthermore attempts to generate a single-celled C4 system have not resulted in a functional C4 cycle in the leaves of a C3 species. The international C4 Rice Consortium has generated resources that will allow it to test the feasibility of generating a functional two-celled C4 pathway in rice. The classic genes encoding C4 metabolic enzymes have been introduced into rice in a cell specific manner, artificial microRNAs are being used to down-regulate part of the Calvin-Benson cycle in the mesophyll cells and C4 transporters required to support the increased metabolic fluxes between sub-cellular compartments of the C4 cycle are also being transformed into rice. These resources are now being combined to install all the genetic components required to create a C4 pathway in rice. An overview of the strategy and timeline for production of C4 rice is presented.

Keyword : C4 Photosynthesis, rice, genetic engineering

PC-16

Overexpression of *nicotianamine synthase* gene in trangenic rice induces the expression of deoxymugineic acid biosynthesis genes and suppresses the expression of *ferritin* genes

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Development of high-iron rice can sustainably reduce the incidence of iron deficiency in populations that do not have easy access to other food options. One strategy to improve iron content in polished rice was to develop IR64 rice events expressing nicotianamine synthase gene *OsNAS2* under the control of constitutive CaMV 35S promoter and soybean ferritin gene *SoyferH-1* under the control of endosperm-specific GluA2 promoter. In this study, events with simple integration of inserted DNA fragment with no DNA transfer beyond borders that showed 8-12 ppm iron concentration of polished seedsunder field evaluation (Trijatmiko et al., manuscript in preparation) were selected and grown in the glasshouse. Transcriptexpression of endogenous genes involved in the deoxymugineic acid biosynthesis pathway, namely *OsNAS1*, *OsNAS2*, *OsNAS3*, *OsDMAS1* and *OsNAAT1*, and ferritin genes *OsFER1* and *OsFER2* were evaluated vis-a-vis the transgenes *OsNAS2* and *SoyferH-1* using quantitative real-time PCR. Analyses were done in the vegetative leaf and mature seed using a subset of 3 high-iron events containing either only soybean *ferritin* or *OsNAS2*, or a combination of both transgenes. Fold change values relative to the wild type show that overexpression of the *OsNAS2* transgene induces the expression of deoxymugineic acid biosynthesis genes and suppresses the expression of ferritin genes.

Keyword : high-iron rice, *nicotianamine synthase*, *ferritin*, real-time PCR, deoxymugineic acid

PC-17

Molecular analysis of putative *flavonoid O-methyltransferases* in rice (*Oryza sativa*)

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Phytoalexins are antimicrobial secondary metabolites accumulated in plant tissues in response to pathogen attacks. Rice produces a wide array of diterpenoid and flavonoid phytoalexins. Sakuranetin is a flavonoid phytoalexin in rice and synthesized by the enzymatic 7-*O*-methylation of naringenin. Accumulation of sakuranetin is induced by pathogen attacks and UV irradiation. Enzymatic methylation of flavonoids is catalyzed by *O*-methylatransferase (OMTs). To identify OMT candidates possibly involved in sakuranetin biosynthesis, molecular characterization of rice OMTs was performed. In rice, thirty OMT genes were found to contain an *O*-methyltransferase family 2 domain which is a protein domain commonly found in flavonoid OMTs. These OMT genes were divided into two groups, 4/7-OMTs and 3'/5'-OMTs by the phylogenetic analysis. Microarray analysis of UV-treated rice leaves was performed to examine the changes of OMT expression in response to UV treatment. The result of microarray analysis showed that the expression of two putative flavonoid OMTs, Os09g17560 and Os11g19840, belonging to a 4/7-OMT genes was up-regulated in response to UV treatment. The induction of Os09g17560 and Os11g19840 gene expression by UV irradiation was also confirmed by RT-PCR analysis. Taken together, these findings suggest that these two genes were OMT candidates potentially participated in the flavonoid phytoalexin biosynthesis in rice under UV stress.

Keyword : Phytoalexin, Sakuranetin, *Flavonoid O-methyltransferase*

PC-18

Characterization of putative rice (*Oryza sativa*) *monolignol β-glucosidases*

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In plants, β-glucosidases belonging to glycoside hydrolase family 1 (GH1) play important roles, such as defense against herbivores, response to biotic and abiotic stresses, activation of phytohormones, and cell wall metabolism. Phylogenetic analysis of rice (*Oryza sativa* L.) GH1 β-glucosidases indicated that Os4BGlu14, Os4BGlu16, and Os4BGlu18 are closely related to known monolignol β-glucosidases, leading to the hypothesis that they are involved in lignification, which may be involved in the response to pathogenic fungi. The cDNAs for each of these rice GH1 β-glucosidase genes, *Os4BGlu14*, *Os4BGlu16*, and *Os4bglu18* were cloned, sequenced, and ligated into pET32a, and the resulting recombinant plasmids were used to express the putative β-glucosidases as fusion proteins with N-terminal thioredoxin and His_6 tags in *Escherichia coli* strain Origami (DE3). No activity could be detected for proteins expressed from *Os4BGlu14* and *Os4BGlu16* in this system. Optimal expression of soluble Os4BGlu18 β-glucosidase activity was obtained with induction at 18˚C, 0.1 mM IPTG for 16 to 18 h. The recombinant protein was extracted and purified by immobilized metal affinity chromatography (IMAC). The optimum pH for Os4BGlu18 was found to be 5 and the enzyme was stable from pH 4 to pH 8. Os4BGlu18 hydrolyzed *p*-nitrophenol (*pNP*)-β-D-fucoside best, followed by *p*NP-β-D-glucoside, *p*NP-α-L-arabinoside, *p*NP-β-D-galactoside and *p*NP-β-D-xyloside, respectively. Moreover, Os4BGlu18 hydrozyed n-octyl-β-glucoside, n-heptyl-β-glucoside, methyl-β-D-glucopyranoside and daidzin. Among possible natural substrates, Os4BGlu18 slowly hydrolyzed the monolignol glycoside *p*-coumaryl alcohol glucoside, along with salicin and arbutin. No activity was detected with coniferin and sinigrin, two other monolignol glucosides, suggesting another isoenzyme may be responsible for their hydrolysis in the plant. To investigate whether expression of these genes is involved in blast resistance, near isogenic lines (NILs) that are resistant and susceptible to blast were grown and inoculated with *Magnaporthe grisea* for 6, 12 and 24 h, 2, 3, 4 and 7 days then analyzed by qRT-PCR. *Os4BGlu16* expression was increased in the resistant NIL within 6 h and at 7 days in the susceptible NIL, while *Os4BGlu18* was mainly increased in the susceptible NIL, but the results were highly variable and need to be verified. Currently investigation of the localization of Os4BGlu14, Os4BGlu16 and Os4BGlu18 and the effects of their over expression and knock down in transgenic rice plants is underway.

Keyword : Rice, lignification, *beta-glucosidase*, *glycoside hydrolase*, protein expression, gene function, fungal infection

PC-19

Natural variations in pseudo-response regulator 37 contribute to rice adaptation to long-day photoperiod at high latitudes

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Heading date (Hd; or flowering time) and photoperiod sensitivity (PS) of crop plants are important traits for fitness in different latitudes in association with crop productivity. Rice is a facultative short-day (SD) plant which flowers early in SD conditions and late in long-day (LD) conditions. However, rice has long been cultivated in a wide range of latitudes through introduction of natural variations in Hd and PS. Especially, elite rice cultivars at high latitudes (40-55^{o}N latitudes) have weak or no PS, and flower early under natural LD (NLD) conditions during short growing season, which is mainly regulated by two quantitative trait loci, *Hd2* and *Hd4*, on chromosome 7. Here, we show that two loss-of-function mutations of *PRR37* (*Hd2*) and *Ghd7* (*Hd4*) are common in the early flowering rice varieties cultivated at high latitudes. *PRR37* acts as a LD-dependent floral repressor by repressing *Hd3a* expression in LD, but no effect on flowering in SD. In this respect, various non-functional alleles in *PRR37* and *Ghd7* are found in many early-flowering japonica rice adapted to high latitude. Our results show that loss-of-functions in both *PRR37* and *Ghd7* enable rice to be cultivated under NLD at higher latitudes.

Keyword : rice, heading date, photoperiod sensitivity, *PRR37, Ghd7*

PC-20

Phenotypic characterization and functional analysis of Ac/Ds mediated mutant pool in Korean rice germplasm

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Rice is the staple food of more than 50% of the world population, and has the small genome size of only 430 megabase (haploid genome), which rendered it a model crop plant for genomic study. Since the sequencing of rice genome was completed by the International Rice Genome Sequencing Project (IRGSP), many researchers in the world have been working to explore the function of genes in rice genome. For functional genomic researches, insertional mutagenesis has been widely used. In maize, well characterized transposable elements have traditionally been used to clone genes for which only phenotypic information is available. In rice, endogenous mobile elements such as MITE and Tos (Hirochika. 1997) have been used to generate gene-tagged populations. Recently T-DNA and maize transposable element systems has been utilized as main insertional mutagens in rice. While T-DNA scheme using Agrobacteria-mediated transformation in rice requires extensive facilities, time, and labor, the Ac/Ds system offers the advantage of generating new mutants by secondary transposition from a single tagged gene. Additionally, revertants can be utilized to correlate phenotype with genotype. To enhance the efficiency of gene detection, advanced gene-tagging systems (i.e. activation, gene or enhancer trap) have been employed for functional genomic studies in rice. This study has been carried out to construct the database for the insertional mutant population generated by Ac/Ds transposable element system. In addition, the biological functions of genes useful for agriculture have been studied and the possibility to create new varieties through the biotechnological method have been exploited. The data and information obtained through this study could be used as a basis for intellectual property and be helpful for breeding to select useful gene as analyzing gene function analysis. This project is performed to develop internationally competitive scale of insertional mutagenized population, and to construct databases of molecular information on Ds insertion sites. Ultimate goals are to supply genetic materials and informations essential for functional analysis of rice genes and for breeding using agronomically important genes.

Keyword : rice, mutant pool, Ac/Ds, phenotypic variation

PC-21

Forward genetics approaches to discover gene controlling amylose content and gelatinization temperature

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The amylose content (AC) and gelatinization temperature (GT) are two major factors that explain cooking and eating quality of rice. To investigate the genes controlled AC and GT, we developed mutant population from fast neutron-irradiation on an italic pericarp Jao Hom Nin (JHN) rice variety. The high throughput screening techniques were used to screen 12,000 M4 lines for both AC and GT. The results showed that 359 lines there was distribution on amylose level from very low (<10%, glutinous) to high (>30%) and some mutant lines mutated to high GT types that strongly differentiated from JHN wild type. The granule bound starch synthase I (GBSSI) and soluble starch synthase IIa (SSSIIa) are key genes known to play influencing in AC and GT respectively. A set of functional markers for GBSSI and SSSIIa was used to survey haplotypes of these mutant lines. The results showed that haplotypes of these mutants were similar to what is found in natural variation. In addition, once extremely high amylose (*Mu3225*, 31%) possesses CT repeat number of 20 $(CT)_{20}$ and found base substitution on exon 6 of *GBSSI* that differ from previous reported. However, the wild variation in amylose content can not be explained by such haplotype variation. A sequence variation of gene involve starch biosynthesis of mutant lines must be further study to identify the gene controlling amylose content and gelatinization temperature.

Keyword: amylose content (AC), gelatinization temperature (GT), *granule bound starch synthase I* (*GBSSI*) and *soluble starch synthase IIa (SSSIIa)*

PC-22

The effects of phytocrome interacting ankyrin repeat protein to the PIF3 phosphorylation

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The phytochrome-interacting factors (PIFs) are common repressor of plant photomorphogenesis in light response. PIFs interact with phytochrome and regulate various light responses. The interaction between PIFs and phytochrome triggers rapid phosphorylation and degradation. Here we show that a phytochrome interacting ankyrin-repeat protein 2 (PIA2) is related to the PIF3-phyA signaling. An in vitro pull-down assay showed that PIA2 interacts directly with PIF3 and phyA. Moreover, the data show that the N-terminal domain of PIA2 suppresses the phyA mediated PIF3 phosphorylation. However phyA induced PIA2 phosphorylation did not affect the interaction between PIF3 and phyA. Further studies showed that helix breaking point mutation to Proline of Arginine13 and Phenylalanine16 residues in N-terminal domain of PIA2 affected to the PIF3-phyA interaction and phosphorylation. Finally, we found that interaction between the PIA2 with PIF3 and phyA regulated anthocyanin biosynthesis. Our results suggest that PIA2 plays a role in the PIF3 phosphorylation and stability during seedling development as well as phytochrome-mediated light signaling in plant.

Keyword : phytochrome interacting, ankyrin-repeat protein2, PIF3 phosphorylation

PC-23

Interacting map and minimal domain of rice *U-box E3 ubiquitin ligases* for specific interactions with E2 enzymes

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Ubiquitination plays a central role in various cellular events in eukaryotes. U-box domain, one of the E3 ubiquitin (Ub) ligase motifs, shows greater number in plants compared to human and yeasts. This divergence in number between species suggests thatU-box E3 ligases might participate in plant-specific events. In ubiquitination, the covalent attachment of Ub to target substrates is crucial. Moreover, specific interaction between E2 and E3 enzymes also contributes in this highly regulated process. Using yeast two hybrid assay, partial binding networks between sixteen Armadilo-repeat (ARM)-containing U-box proteins and UBC fold of thirty E2s in rice were predicted. Subsequently, to define the minimal domain of U-box that is needed for binding to E2s, yeast two hybrid assay was again performed using various deletion mutants of rice ARM-containing U-box proteins and specific E2 proteins. A full-length U-box motif or deletion mutants without one of the N- or C-terminal extensions failed to interact with E2 protein, suggesting that both N- and C-terminal extensions were required for the specific interactions between E3s and E2s. Interestingly, the length of extension required in interaction with E2s was different between different classes of U-box proteins. Our data imply that both N- and C-terminal extensions of U-box have a critical role in interacting with E2 enzyme. This work was supported by grants from the Next Generation BioGreen 21 Program (Project No. PJ008152) and the NRF (Project No. 2010-0000782) to WTK. HJM was recipient of BK21 scholarship.

Keyword : ubiquitination, E2, E3, u-box, Y2H

PC-24

Targeted *SSS4A* gene mutagenesis using *Zinc finger nucleases* (*ZFNs*) in rice genome

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Zinc finger nucleases (*ZFNs*) have been used for targeted mutagenesis in eukaryotic cells. Custom-designed ZFNs can induce double-strand breaks (DSBs) at a specific locus. Our custom ZFN dimer was designed 3-finger of left and 4-finger of right with 2 kb size using 2A. A Ti-plasmid vector, pTA7002 containing the target site of *SSS4A* gene for a ZFN pair, that was shown to be active in yeast, was integrated in the rice genome. This promising technique for genome engineering was induced into 4 exon region of *SSS4A* gene in rice genome using *Agrobacterium*-mediated transformation. The *SSS4A* full-length cDNA was 5,070 bp consisting of a 318 bp 5′-untranslated region (UTR), a complete ORF of 2,928 bp encoding a polypeptide of 975 amino acids and a 3′-UTR of 1,824 bp. The vector is based on glucocorticoid receptor inducible gene expression system. Thus, *SSS4A::ZFN* expression was tightly controlled and the phenotype in low concentrations 10uM of the glucocorticoid hormone dexamethasone (DEX). In plant cells, transient ZFN expression is achieved by direct gene transfer into the target cells. For an alternative, ZFN delivery and production of mutant plants using a tobacco transient expression system for indirect transient delivery of ZFNs into a variety of tissues and cells of plants. ZFN activity was determined by PCR and sequence analysis of the target site. ZFN induced plants were obtained in up to 2% of the PCR products, consisting of deletions ranging between 1and 100 bp and insertions ranging between 1 and 10 bp. Our results describe an alternative to direct gene transfer for ZFN delivery and for the production of mutated rice.

Keyword : *Zinc finger nucleases (ZFNs),* glucocorticoid hormone dexamethasone (DEX), targeted mutagenesis

PC-25

Molecular characterization of *BrUGE1* encoding *UDP-glucose-4-epimerase* in rice

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UDP-glucose 4-epimerase catalyzes the reversible conversion of UDP-glucose to UDP-galactose. The gene, named *BrUGE1,* isolated from a Chinese cabbage had a total length of 1,328 bp that contains a single open reading frame (ORF) of 1,056 bp which encodes a polypeptide of 351 amino acid residues with a calculated mass of 39.0 kDa. Sequence analysis of *BrUGE1* protein has the characteristic of an active site tetrad and NAD-binding motif (typically TGXXGXXG) of the extended short chain dehydrogenase/ reductase (SRD) superfamily. Expression analysis showed that *BrUGE1* is tissue specific and highly expressed in stem of rice plant. Interestingly, *BrUGE1* mRNA was highly accumulated by drought stress with significantly higher amount of soluble sugar. Morphological evaluation showed an increase in yield by 27%. Panicle length, number of productive tillers/hill, and filled spikelets were significantly increased by 17~20% compared to the wild type Gopum. Moreover, the growth of the wild type Gopum seedlings on galactose was increasingly inhibited with a decrease in UDP-glc epimerase 1 expression compared to the transgenic rice lines. In the *Ubi-1*::*BrUGE1* lines, the increase of UDP-glc epimerase 1 expression was apparently sufficient to overcome the toxic effects of galactose. Taken together, the *Ubi-1*::*BrGUE1* rice lines increased yield probably by increasing the rate of filled grains. The enhanced drought tolerance may be due to the induction of soluble sugar which may act as osmolyte to compensate dehydration during drought stress.

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Keyword : *BrUGE*, soluble sugar, abiotic stresses, rice

PC-26

Characterization and fine-mapping of a shortened uppermost internode mutant in rice

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We found a mutant rice line that has an extremely shortened uppermost internode in the Ac/Ds insertional mutant population and named it *sui4*. We crossed the mutant type plant with its original variety, Dongjin. The F1 plant showed intermediate phenotype, and F2 plants were segregated into normal, mutant, and intermediate types, which indicated that *SUI4* is an incomplete dominant gene. This trait was not co-segregated with Ds element, and we crossed this mutant line with Milyang23 to carry out mapping of the *SUI4* gene. We produced 273 F2 pants and found that the *SUI4* gene is located on the chromosome 7 by the bulked segregant analysis (BSA). Primary mapping revealed that this gene is located in the interval of about 4.5Mb flaked by markers of *S07010* and *S07015*. Further fine mapping with F3 lines derived from F2 plants that have recombination in this interval narrowed down the*SUI4* region to 1.1 Mb interval flanked by *RM1253* and *S07015*.

Keyword : rice, mutant, internode, mapping

PC-27

The rice protein kinase *OsPSTOL1* is an enhancer of root growth

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Phosphorus (P) is an essential macro element for agricultural production systems. However, P is not easily available to plants since it may be fixed in the soil and phosphate rock, the source of P-fertilizer, is a non-renewable, limited resource. The identification of the major rice quantitative trait locus (QTL) *Phosphorus Uptake 1* (*Pup1*) which confers tolerance to P-deficiency was a first significant step towards the development of P-efficient rice varieties. After the function of the *Pup1* locus could not be directly linked to P uptake, sequencing of the *Pup1* locus in the tolerant donor parent Kasalath now revealed the presence of a novel Ser/Thr protein kinase gene, *PHOSPHORUS STARVATIONTOLERANCE 1* (*OsPSTOL1*). This gene is located in a Kasalath-specific insertion-deletion (~90kbp) region which is absent from the Nipponbare reference genome and other intolerant rice varieties. Overexpression of *OsPSTOL1* in two varieties that naturally lack the gene conferred tolerance to P deficiency, as demonstrated by increased plant biomass, P content, and grain weight. *OsPSTOL1 is* specifically expressed in root primordia and, in agreement with that, transgenic plants and *Pup1* near-isogenic lines show early vigorous root growth. This enables plants to forage a larger soil area for P and other nutrients. Gene specific molecular markers were developed that are now being used for molecular breeding of P-deficiency tolerant rice varieties.

Keyword : phosphorus, protein kinase, root growth, abiotic stress, QTL

PC-28

Enhanced saccharification of rice straw by senescence-induced expression of cellulase.

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Efficient production of carbon-neutral biofuels is key to resolving global warming and exhaustion of fossil fuels. Cellulose, which is the most abundant biomass, is physically strong and biochemically stable, and these characteristics lead to difficulty of efficient saccharification of cellulosic compounds for production of fermentable glucose and other sugars. To overcome this problem, we showed that constitutive overexpression of cellulase enhanced saccharification of rice straw, but the overexpressing rice plants also showed various morphological and physiological defects and sterility. To avoid these problems, we examined reliability of a promoter of a senescence-inducible gene instead of the constitutive promoter. First, we examined the promoter activity of *STAY GREEN* (*SGR*) of rice, which is known to be expressed along with senescence. The *SGR* promoter induced senescence-specific expression of the GUS reporter gene in rice. Next, we generated transgenic rice plants with various cellulase genes under the control of the *SGR* promoter. In these plants expression of the cellulase was induced along with senescence, and these plants showed no morphological abnormarity or sterility. Furthermore, they showed enhanced saccharification of their straw. Our results indicate that genetic engineering of cellulosic biomass plants by senescence-induced expression of cellulase genes will be one of the approaches to confer enhanced saccharification ability for efficient production of cellulosic biofuels such as ethanol.

Keyword : Cellulase, Biofuel, Senescence-inducible promoter

PC-29

Detection of mutations in regenerated rice by whole-genome sequence analysis

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The technology of next-generation sequencing (NGS) is enables to obtain the whole-genome sequence of individual plant. For the improvement of crops, especially for the marker assisted breeding, discovery of DNA markers is essential. The efficient method to detect polymorphism from the NGS data is required. We have developed 50,000 lines of regenerated rice for making mutant panel by *Tos17* insertions. We already analyzed insertion points of transposed *Tos17* in the rice genome and created the database. Half of mutant lines showed at least one phenotype. The difference of phenotype of regenerated plant is called 'somaclonal variation'. Although the high frequency of the visible phenotypes was observed in the regenerated rice, contribution of gene disruption for the phenotype by *Tos17* is estimated less than 10%. To understand of the somaclonal variation, we sequenced some regenerated lines and analyzed the NGS data using the original pipeline for mutation detection. We found the mutation rate of regenerated rice is accelerated. We also found structural change of genome including a large segmental duplication, small deletions in addition to transposition of *Tos17*. These polymorphisms will be useful for functional analysis of rice genes. And also, our techniques for mutation detection will contribute for molecular breeding of crops.
Miyao, A., *et al.* (2012) Plant Cell Physiol. 53:256-264.

Keyword : Tos17, somaclonal variation, SNP

PC-30

Detection of copy number variations in the rice genome using next-generation sequence data

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Copy number variations (CNVs) are increasingly acknowledged as an important source of evolutionary novelties. In human, various CNVs have been detected related to diseases. Little is known, however, about the contribution of CNVs to genetic variation in rice. With the availability of the resequence data of 50 accessions of rice, we attempt to determine the extent and functional impact of CNVs in rice. Three major approaches were used to identify CNVs in rice: paired-end mapping BreakDancer, split-read analyses Pindel, and read-depth analyses CNVnator. Calls detected by two or more algorithms (50% reciprocal overlap) are regarded as high confidence. All the confident calls refined by local assembly were filtered by calls less than 3 accessions. Finally, our dataset encompassed 9207 deletions, 614 insertions and 87 inversions. For the deletions data, we assessed the putative functional impact of CNVs further by relating them to genome annotation. As expected, the majority of CNVs are TE-related (5045) and no-annotation (3008), suggesting that CNVs are enriched for sequences with nonessential functions. There are 1675 rice genes affected by CNVs. By gene ontology analysis, we observed significant enrichment for several functional categories, including defense response, and response to stress. This finding is consistent with these genes being particularly diverse because of pathogen pressure. Next, we will seek to compare the structural polymorphisms in population and study selective pressure upon structural variations. In addition, by comparing the CNVs with dbSNP data from the same population, functional consequences of CNVs will be defined.

Keyword : copy number variation, rice, resequencing

PC-31

RiceVarMap: a comprehensive database of rice genomic variations

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We constructed a database of rice genomic variations, Rice Variation Map (RiceVarMap, http://ricevarmap.ncpgr.cn). The database provides comprehensive information of 6,551,358 single nucleotide polymorphisms (SNP) and 1,214,627 insertion/deletions (INDEL) identified from low-coverage sequencing data of 1,479 rice varieties. The genomic variations were annotated based on MSU Rice Genome Version 6.1. The SNP genotypes of all varieties were imputed and evaluated, resulting in a missing data rate less than 1% and an estimated accuracy greater than 99%. The SNP genotypes of all varieties can be queried. In particular, the database provides a tool to compare any two cultivars and find out genomic variations between them. For each SNP/INDEL, allele frequencies within various sub-population and the effect of the variation are also listed. Users can search SNP/INDELs by identifiers of the SNP/INDELs or genomic regions, then filter SNPs by allele frequencies. Users can also identify large effect genomic variations that may alter the protein sequence of a gene by searching SNP/INDELs using the gene identifier as keyword. Moreover, the query results are available for users to download through our website. Such information is expected to be useful for identifying genetic variations and genetic improvement in rice.

Keyword : Genetic variation, Database, SNP, Genotype, INDEL

PC-32

Comprehensive analysis of genetic polymorphisms in Korea rice accessions by whole genome resequencing

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Recent advances in whole genome sequencing have allowed us to identify genes associated with agronomical important traits, molecular-assisted breeding and quantitative trait locus (QTL) in crop species. Now we obtained 2,447 million raw reads which provided over 58x fold-coverage of the Nipponbare genome by sequencing whole genome of ten Korea rice accessions. After mapping a large number of short reads from ten accessions onto Nipponbare sequence from IRGSP build 7.0 as reference genome, we detected 3,144,016 SNPs which estimated to be one per 2.2Kb on average. Among 553,527 SNPs in cording region (17.6% of total SNPs), 287,386 SNPs were non-synonymous SNP (1.69 SNPs/gene). 9,040 genes were not found SNP in all ten accessions at all. The cultivar specific SNPs were examined to analysis of correlation between the specific agronomical traits and variation of genomic sequence in each accession. It accounted for 1~12% of the total SNPs and approximately 15% of cultivar specific SNPs were located in genic region. And by unmapped region against the reference genome, we observed 8,152 possible mRNA loss events. We also found that 286 mRNAs of 8,152 lost mRNAs were recovered in unknown chromosome and 749 mRNAs were located on both 12 chromosomes and unknown chromosome in duplicate. These variants obtained from our results should be valuable to identify agronomically important genes and molecular marker for rice breeding.

Keyword : rice, SNP, polymorphism, genome resequencing

PC-33

Differences of folate biosynthesis gene expression in colour and colourless husked rice grain

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Although the consumption of food and vitamin supplements has dramatically increased worldwide, vitamin deficiency is still a problem in many countries. Folate or vitamin B9 plays various important roles in methylation process, nucleic acid and amino acid biosynthesis and other one-carbon metabolism pathways which helps maintaining of normal organ functions. Folate deficiency can cause the retardation of cell growth and affect folate-mediated organ functions. As rice is a major part of the Asian and African diets, the development of high folate rice varieties can be the long term solution of folate deficiency in these regions. Current study focused on the differences of 5 folate biosynthesis gene expression—GTP cyclohydrolase I (*GTPCHI*), aminodeoxychorismate synthase (*ADC synthase*), hydroxymethyldihydropterin pyrophosphate kinase/dihydropteroate synthase (*HPPK/DHPS*), folypolyglutamate synthase (*FPGS*) Os03g02030 and *FPGS* Os10g35940—in 15 colour and colourless husked rice varieties. Quantitative real-time PCR was performed significant between samples. The highest expression of *GTPCHI*, *ADC synthase* and *HPPK/DHPS* genes was detected in RD29, RD33 and KKU ULR 229 varieties which implies the highest amount of tetrahydrofolate monoglutamate in these 3 varieties. Moreover, the colour hasked rice varieties exhibited the higher expression of FPGS Os03g02030 and FPGS Os10g35940 genes than the colourless hasked rice varieties which tetrahydrofolate polyglutamate tends to be much more produce in the colour husked rice grains.

Keyword : rice, folate, vitamin deficiency, gene expression

PC-34

Expression of QTL based and other candidate genes in Madhukar x Swarna RILs with contrasting levels of iron and zinc in unpolished rice grains

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Identifying QTLs/genes for iron and zinc in rice grains can help in biofortification programs using non transgenic approaches. Genome wide mapping showed 14 QTLs for iron and zinc concentration in unpolished rice grains of F7 RILs derived from Madhukar x Swarna (Gene 508: 233–240, 2012). High priority candidate genes underlying QTLs were *OsYSL1* and *OsMTP1* for iron, *OsARD2, OsIRT1, OsNAS1, OsNAS2* for zinc and *OsNAS3, OsNRAMP1*, *Heavy metal ion transport* and *APRT* for both iron and zinc. One line (HL) with high Fe and Zn (123 ppm Fe and 102 ppm Zn) and one line (LL) with low Fe Zn (2 ppm Fe and 22 ppm Zn) in unpolished rice were compared with Swarna (22.5 ppm Fe and 27.2 ppm Zn) as control for gene expression in shoots and roots using qPCR. 15 days old seedlings were grown in Fe- and Fe+ medium to identify genes differentially expressed in Fe- conditions. *OsNRAMP1* was up-regulated in all samples in Fe- but more in LL. *OsNAS1, OsNAS2, OsIRT1, OsZIP6* and *OsZIP8* were up-regulated in both root and shoot in all except in Swarna shoot. *OsNAS3* and *OsYSL1* were up-regulated in all but down-regulated in Swarna root and shoot. Expression of *OsNAS1, OsNAS2, OsNAS3, OsIRT1, OsYSL1* and *OsZIP8* was increased in shoots of HL and roots of LL. Expression of *OsZIP6* was more in HL than in LL or Swarna. Under Fe deficiency, *OsYSL15* was up-regulated 58-fold in shoots of HL and *OsYSL2* was expressed only in roots of Swarna and LL indicating a major role of these two genes.
The work was financially supported by Indian Council for Agricultural Research, Govt. of India grant NPTC/FG/05/2672/33-3019 to NS.

Keyword : biofortification, iron, zinc, qPCR, Fe deficiency

PC-35

Overexpression of rice *Nicotianamine synthase2* and soybean *FerritinH1* in transgenic rice alters the expression of endogenous iron acquisition genes

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Rice endosperm lacks essential micronutrients notably iron. Iron deficiency leads to iron deficiency anemia (IDA), which may cause morbidity, impaired mental development, and death. At IRRI, rice transgenic events with high iron concentration in starchy endosperm harboring selected iron acquisition genes are being characterized. Gene expression at the reproductive stage in the flag leaf and seed endosperm at developmental stage of maximum iron loading (milky and dough) of the selected high-iron concentration (12-18 ppm) transgenic event (Trijatmiko et al., in preparation) expressing OsNAS2 and SoyFERH1), and its wild-type parent IR64 were determined by relative real-time quantitative PCR. The 31 targeted genes are the endogenous genes involved in iron uptake and translocation (OsDMAS, OsFRO, OsIRT1, OsNAS, OsNAAT, OsTOM, and OsYSL), storage (OsFER), compartmentalization (OsVIT1, OsNRAMP), regulation (OsIRO), divalent metal transport (OsZIP), and the two transgenes (OsNAS2 and SoyFERH1).For each gene, fold change in target gene expression level relative to the control parent IR64 was calculated using the 2^{-DDCt}method (Livak and Schmittgen, 2001). All expression data (Cp values) were normalized against the geometric mean of two rice endogenous reference genes- triose phosphate isomerase and tumor protein homolog. Expression of the iron acquisition genes in the transgenic high-iron line increased, most significantly for the key iron transporter genes YSL4, YSL15, YSL18, and FRO2, and for the iron deficiency response regulator gene IRO2. These genes, alone or in combination, could be good targets for engineering rice transgenics for increasing iron content sufficiently to satisfy human iron requirements.

Keyword : high-iron rice, real-time PCR, iron acquisition, delta delta Ct method, transgenic

PC-36

Transformation and expression of a gene controlling anthocyanin biosynthesis from Arabidopsis in rice

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The *pap1* (production of anthocyanin pigment) gene isolated from *Arabidopsis thaliana* encodes MYB transcription factor which controls anthocyanin biosynthesis. In this study, *pap1* gene was transformed into rice CV. Kitaake by *Agrobacterium* method. We investigated function of *pap1* gene and its effects on expression of structural genes involved with anthocyanin biosynthesis in rice. Three transformed rice plants were analyzed by Semi-quantitative RT-PCR and Real time-PCR techniques. All analyzed plants showed similar expression levels of *pap1* gene. The structural genes, *CHI, CHS, F3¢H* and *DFR*, were expressed in transformed riceplants similar to those of untransformed plants. *ANS* gene was not expressed in both transformed and untransformed rice plants. The results showed that *pap1* gene did not promote the expression of structural genes in transgenic rice plants. Although, these transformed rice plants showed expression of *pap1* gene from *Arabidopsis* which is a dicot, the gene could not affect expression of structural genes in anthocyanin biosynthesis pathway in rice. Possibly regulatory mechanism of anthocyanin biosynthesis in monocots may be different from that of dicots.

Keyword : rice, pap1 gene, anthocyanin, *Agrobacterium tumefaciens*

PC-37

Evaluation of high iron rice expressing *OsYSL2* transporter gene

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Cereals, especially rice, are a poor source of iron. Increasing the content of bioavailable iron in rice endosperm through transgenic technology offers an opportunity to develop biofortified-rice to lessen the prevalence of iron deficiency in the developing countries. *OsYSL2* transporter gene, a critical Fe-nicotianamine transporter, was successfully introduced into high iron breeding line (IR69428) driven by two phloem-specific promoters: OsSUT1 and OsTRXh. The Inductively Coupled Plasma by Optical Emission Spectrometry (ICP-OES) analysis revealed that polished T1 rice seeds of single copy transgenic events harboring *OsYSL2* gene driven by OsTRXh generated higher iron concentration up to 7.3 mg/kg as compared with events containing *OsYSL2* gene driven by OsSUT1. Perl's Prussian blue staining method indicated high iron accumulation in the aleurone layer of dehulled T1 seeds, as well as in other parts of grain. Approximately, 40% of the transgenic events with OsTRXh showed intense blue coloration compared to 27% of the transgenic events with OsSUT1. *In silico* analysis (using RiceXPro database; http://ricexpro.dna.affrc.go.jp/) of the *OsSUT1* and *OsTRXh* genes indicated extensive expression of the two genes in vegetative tissues (i.e., leaves and stems). Furthermore, it is predicted that OsTRXh has a more conserved expression pattern compared to OsSUT1 throughout the plant development. Preparation of prOsTRXh::GUS construct is underway for more detailed analysis of the promoter expression pattern. This study suggests the involvement of *OsYSL2* transporter in the long distance transport and accumulation of iron in the rice endosperm when driven by the OsSUT1 and OsTRXh promoters.

Keyword : *OsYSL2, OsTRXh, OsSUT1*

PC-38

Reverse and forward genetics approaches to identify mutants conferring high grain Fe density and tolerance to Fe toxicity

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Rice contains the lowest grain Fe content among important cereal crops. One such limiting factor is related to the variation in tolerance to Fe toxicity. Reverse and forward genetics approaches were used for identification of high grain Fe density and Fe toxicity tolerance in the M_4 TILLING population created by fast neutron. One successful reverse screening was the identification of FRO mutant which accumulates 21-30 % more grain Fe content than its wild type with high Fe toxicity tolerance for seedling in 300 ppm solution. More successful screening was done using Perl Prussian Blue (PPB). Among 12,000 M_4 screened so far, 76 mutants contained higher Fe grain density while only 2 mutants were very low. The Fe grain density was varied from 29 ppm (JHN 4643) to 7 ppm (JHN 8097). On the other hand, screening for seedling Fe tolerance at 300 ppm toxic level in the same 12,000 M_4 mutant lines identified 95 tolerance and 57 susceptible lines compared to the wild type. In order to rapidly identify mutation hotspot coding regions, genome-wide screening for single feature polymorphism (SFP) was conducted using 57K Rice GeneChip expression array. Using 30% FDR, 45 SFPs were distinct in JHN 4643 and 32 SFPs in JHN 8097 from the wild type. Confirmed by sequencing, newly SNPs were identified cation transporters including PDR-like ABC transporter, Zinc finger, Indole-3-glycerol phosphate lyase, and Chlorophyll a oxygenase. In the low Fe JHN 8097, the Chlorophyll a oxygenase was interrupted by 28 bp compared to the wild type. Unexpectedly, the 28 bp insertion was identical to a conserved motif found in other three oxidoreductase genes closely linked on chromosome 3. The $F_2$and $F_{2:3}$ segregating populations generated from the cross between JHN 4643 and JHN wide type revealed a single Mendelian segregation for grain Fe content. Identification of the candidate gene for low Fe grain density is now on-going. Such gene may play important roles in Fe uptake from soil or Fe transportation to grains.

Keyword : Rice, mutants, iron density, iron toxicity

PC-39

Involvement of ubiquitin-mediated protein degradation machinery during sporophyte-to-gametophyte (S2G) transition in rice anthers

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Life cycle of flowering plants alternates between a dominant sporophytic phase, and a shorter gametophytic phase represented by pollen and the embryo sac. The reductional division (effected during meiosis) in a set of specialized sporophytic cells (pollen mother cells) of developing anthers marks this transition and acts as a two-way-switch that results in specific activation as well as inactivation of a large sub-set of genes in the gametophyte. A large proportion of genes involved in this transition have not been assigned any function but of the known genes, a large number shows affiliation to the ubiquitin-mediated protein degradation machinery. The components that require special mention are F-box and SKP genes, which impart target recognition specificity to the E3-ligase (SCF) complex that ubiquitinates specific protein destined for degradation by the 26S proteasome complex. From a detailed microarray based transcriptome analysis, we have identified a set of rice F-box and SKP-like genes that express in a sporophyte-to-gametophyte (S2G) transition specific manner. Preliminary experiments have revealed specific interactions between these two components indicating possible existence of S2G transition-specific targets of the ubiquitin-mediated protein degradation machinery that may play important roles during transition of the sporophytic to the gametophytic phase of life cycle in plants.

Keyword : E3-ligase, F-box, Gametophytic development, Meiosis, Pollen

PC-40

ETERNAL TAPETUM 1 promotes tapetal cell death during male reproductive development in rice

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Programmed cell death (PCD) is essential for the development of multicellular organisms, yet pathways of plant PCD and its regulation remain elusive. Here we report that ETERNAL TAPETUM 1 (EAT1), a bHLH transcription factor conserved in land plants, positively regulates PCD in tapetal cells in rice anthers. *eat1* exhibits delayed cell death in the tapetum and has aborted pollen development. Expression and genetic analyses revealed that EAT1 acts downstream of TDR, another positive regulator of tapetal PCD. EAT1 can also interact with TDR. This work reveals a dynamic regulatory cascade in male reproductive development in rice, in which EAT1 acts in a downstream branch of the TDR-mediated pathways as an executioner of PCD and simultaneously works together with TDR in regulating other aspects of anther development.

Keyword : Male reproductive, PCD, bHLH

PC-41

Microspore and tapetum regulator 1 encodes a secretory Fasciclin glycoprotein required for male reproductive development in rice

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In flowering plants, formation of the haploid male gametophytes in anthers requires the interaction between reproductive cells and the neighboring somatic cells, yet the underlying mechanism remains poorly understood. Here, we reveal the crucial role of a fasciclin glycoprotein, MICROSPORE AND TAPETUM REGULATOR1 (MTR1), in controlling the development of sporophytic and reproductive cells in rice (*Oryza sativa*). *MTR1* is specifically expressed in the male reproductive cells, yet its mutant exhibits defects in both tapetum and microspore development, causing complete male sterility. We also demonstrate that the fasciclin domains, N-glycolation, and N-terminal signal peptide-mediated plasma membrane localization of MTR1 are required for normal anther development and pollen fertility. Our findings provide the first example that a gene expressed in the male reproductive cells plays a role in coordinating the development of reproductive cells and their adjacent somatic cells, offering significant insights into the mechanism of plant male reproductive development.

Keyword : rice, anther, fasciclin glycoprotein

PC-42

Microsatellite markers: A tool for the validation of true mutants in mutation breeding programme in rice

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Phenotypic selection is the most widely used and practical method in many breeding programs, including in the selection of mutants in mutation breeding in rice (*Oryza sativa*). However, if the desired trait to be selected is the one that can be found readily in natural population, differences found in the mutant population may not always be said as mutants. Here, we showed the possibility of field hybridization or out-crossing as another source of phenotypic differences leading to false-positive results. A set of 24 SSR markers was used as a quality control in the analysis of putative maturity mutants and selected from mutant populations. Results indicated that SSR could be used as a quality control tool in the validation and differentiation of true mutants from possible out-crossed ones.

Keyword : mutation breeding, rice, out-crossing, SSR markers, true mutants

PC-43

Analysis of mitochondrial genes causing pollen abortion and restorer of fertility genes in *Oryza rufipogon*

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Cytoplasmic male sterility (CMS) is one of the most ideal phenomena known in flowering plants to describe the incompatibilities between mitochondria and nuclear genomic interactions. We compared mitochondrial genes causing pollen abortion and nuclear-encoded restorer of fertility genes rescuing pollen from incomplete development in wild rice (*Oryza rufipogon*) and the most characterized BT-type CMS. In BT-CMS, a causative mitochondrial gene for CMS is *orf79* co-transcribed with *atp6* and a rescuing nuclear gene is *RF1a* encoding a RNA binding protein, pentatricopeptide repeat (PPR) protein. We found sequence variants of *atp6-orf79* and highly variable*Rf1a* alleles in wild rice accessions, W1084, W1112. Although an *Rf1a* allele contained premature stop codon in W0133, we found presumed functional *Rf1b* (another *Rf* gene of BT-CMS) in W0133. We presume, in W0133, instead of non-functional *Rf1a, Rf1b* plays a role of the potential restorer of fertility genes in W0133-CMS. Our hypothesis is that different *Rf* alleles are evolved to interact with respective mitochondrial genes in a gene-for-gene fashion. The wild rice accessions used in this study were distributed from the National Institute of Genetics supported by the National Bioresource Project, MEXT, Japan. This study is supported by a Grant-in-Aid (No. 24117502) from MEXT, Japan

Keyword : CMS, *Rf1*, PPR,

PC-44

A label-free quantitative shotgun proteomics analysis of rice pollen development

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Although a great deal of rice proteomic research has been conducted, there are relatively few studies specifically addressing the rice pollen proteome. Rice pollen is sensitive to temperature changes thus low and high temperature cause sterility of panicles. Here, we performed comparative shotgun proteomic analysis of rice pollen development to construct an in-depth proteome reference map, monitoring the expression patterns of the identified proteins, and to detect proteins that are expressed differentially during pollen development. Anther were harvested in 20 days before flowering, 10 days before flowering, and flowering day. The protein expression patterns were revealed by a quantitative shotgun proteoemic analysis. Extracted proteins were separated in 1D-SDS-PAGE then the gel was sliced into seven. Chopped gels were ingel-digested. Peptides were subjected to mass spectrometry (Q-Exactive) ms/ms spectra were analyzed through Proteome Discoverer. By merging all of the identified proteins in the seven sliced gel samples, total anther proteome reference map was constructed. The total spectra counts assigned to a protein were used for quantification. Proteins expression patterns were investigated by comparing the quantity of protein. The proteins expression patterns were identified in this study were clustered. Clustering analysis and Genome Ontology category enrichment analysis revealed that proteins involved in the metabolic process were enriched through all stages of development. With a label free shotgun proteomic approach, this is the report conducting comprehensive identification of rice anther proteins

Keyword : Shotgun Proteomics, Anther, Pollen

PC-45

Analysis of gene expression in anther of thermo-sensitive genic male sterile rice (TGMS) using cDNA-AFLP

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This research studied gene expression in anthers of temperature sensitive genic male sterility (TGMS) rice lines in fertile (low temperature) and sterile (high temperature) conditions using cDNA-AFLP. Two TGMS rice lines were used. These lines have different genetic backgrounds and are controlled by different *tgms* genes. Concordantly between the two TGMS lines, cDNA-AFLP fingerprinting using 30 primer-combinations showed 124 different transcript-derived fragment (TDFs), in which 44 TDFs were expressed under high temperature condition but were not expressed under low temperature condition, and 80 TDFs were expressed under low temperature condition but were not expressed under high temperature condition, A total of 64 TDFs were cut, purified and sent for sequencing but only 24 sequences were obtained. Using Blast search in Gremene rice database, these sequences showed significant homology to 14 genes. These genes were selected to confirm the expressions by Reverse - Transcriptase Polymerase Chain Reaction (RT-PCR), resulting in identification of two groups of genes probably directly involved in TGMS phenotype of rice plants. The first group has 3 genes showing high expression in low temperature and no expression in high temperature conditions in both TGMS lines, but these genes showed high expression in high temperature and low expression in low temperature conditions in wild-type rice plants. The second group was only one gene having opposite patterns of expression from the first group. The information gained from this study could be useful in rice breeding programs for TGMS and for adaptable lines to temperature changes in the future.

Keyword: cDNA-AFLP, *Oryza sativa*, Temperature sensitive, genetic male-sterile

PC-46

OsMADS32 determinates rice floral organ identity by genetic interacting with *OsMADS1*, *OsMADS58* and *OsMADS13*

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Rice reproductive organs provide staple food for human. Therefore, research on uncovering the molecular regulatory network of rice flower organ development is necessary for both theoretic and practical application. In this study, we characterized a mutant with palea and lodicules growth defects, which is caused by lose of function in a grass-specific homeotic gene OsMADS32. Interesting, *osmads32osmads1*, *osmads32osmads58* and *osmads32osmads13* doubl e mutants phenotype analysis revealed that*OsMADS32* could genetically interact with Os*MADS1*, *OsMADS58* and Os*MADS13* in controlling stamen and carpel development, which was further supported by the yeast two-hybrid experiment that OsMADS32 protein could interact with OsMADS1, OsMADS58 and OsMADS13 in vitro. These results implied that *OsMADS32* might be an E-like gene in controlling rice palea, lodicules, stamen and carpel identity

Keyword : Rice, flower organ, *OsMADS32*

PC-47

Rice *ORMDL* is involved in male sterility by affecting pollen development

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Application of temperature-sensitive genetic male sterility (TGMS) system provides a great potential for improving food production by hybrid rice. We identified a deletion of 70 kb in chromosome 7 in TGMS lines controlled by *tms2*, and developed gene-specific markers flanking the deletion for selecting the *tms2* gene. By genotyping several rice species and sub-species, a gene specific marker located inside the deletion area was shown to be a rare allele present only in the *tms2*. Using panicle and pollen of wild-type plants, expression analysis of 19 genes located near and inside the deletion showed that only *ORMDL* gene exhibited different levels of expression in low and high temperatures. RNAi transgenic rice plants having low expression of the *ORMDL* were sterile, and had abnormal pollens. Our results indicated that plant *ORMDL* is involved in male sterility and changing expression of this gene affecting pollen development.

Keyword : *ORMDL*, male sterility, RNAi, TGMS, Marker assisted selection

PC-48

\Transcription factors involved in ammonium assimilation and root growth in rice plants

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Ammonium ion is the major nitrogen in rice paddy soils and is utilized as the major source for N-assimilation in rice crops. In roots, the expressions of ammonium uptake and assimilation-related genes are highly sensitive to the availability of exogenous ammonium. In this study, the function of *OsIDD10* (*Oryza sativa* indeterminate domain 10) has been explored in rice plants. Compared to wild-type roots, *idd10* mutant roots are hypersensitive to exogenous ammonium. IDD10 activates the transcription of *AMT1;2* and *GDH2* by binding to a *cis*-element motif present in the promoter region of *AMT1;2* and in the fifth intron of *GDH2*, respectively. In order to understand the relationship between ammonium-related physiological processes and root growth, transcription factor genes whose expressions are highly sensitive to ammonium have been screened with rice DNA microarray. Nearly 100 transcription factor genes have been selected that showed either at least 5 fold induction or 2 fold suppression in roots in 3 hrs after being exposed to ammonium. T-DNA insertional lines are being screened to identify mutants of these ammonium-responsive genes. Also, phenotypes of mutants are being examined to determine whether these genes are related to root growth and nutrient response. Mutants of one candidate gene that encodes a zinc finger protein, showed short root phenotype. The ultimate goal is acquisition of molecular tools to enhance nitrogen utility efficiency of crop plants and subsequently to increase crop yield for human welfare.

Keyword : ammonium, roots, transcription factors

PC-49

Formation of the late inner root cortex layer involves *OsSCR2* and *OsSHR*, GRAS transcription factors orthologs of *Arabidopsis thaliana AtSCR* and *AtSHR* genes.

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Optimal root development is central for plants to reach maximum growth and yield. Most of our knowledge regarding genes involved in root development has been accumulated in the dicotyledon model plant *Arabidopsis thaliana*. Roots of rice, the monocotyledon model, present several extra ground tissue layers compared to *Arabidopsis*. Between epidermis and endodermis, rice possesses two outer cell layers, exodermis and schlerenchyma, and a multilayered cortex. Variation in the number of cortex cell layers depends on the rice root type and cortex is one of the key tissues for rice adaptation to submergence and tolerance to other environmental stresses.Cortex formation in rice is a two-step process. Most of the cortex cell layers are formed early during root primordia differentiation. Several days after root primordia emergence, a late cortex layer becomes visible. Cortex and endodermis differentiation in *Arabidopsis* has been extensively studied during the last 10 years. A gene network consisting of transcription factors, hormonal and epigenetic pathways have been described. In a first attempt to identify key genes involved in cortex formation in rice, we characterized rice orthologs of *AtSCR*and *AtSHR,* two GRAS transcription factors involved in this developmental process in *Arabidopsis*. First results demonstrate that *OsSCR2*and *OsSHRs* are involved in the formation of the late cortical cell layer. Like in *Arabidopsis*, *OsSCR2* and *OsSHRs* regulate endodermis cell layer division to form news cortex layers.

Keyword : Root, *OsSCR, OsSHR*, Ground Tissue.

PC-50

AZORIZ : specificity of the phytostimulatory cooperation between *Azospirillum lipoferum* and rice

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The establishment of mutualistic or pathogenic associations usually involves partner recognition and requires a specific molecular crosstalk between the plant and the invading microorganism. By comparison, the associative symbiosis (cooperation) between crop plants and PGPR bacteria (plant growth-promoting rhizobacteria) are considered as "simple" interactions, involving low or no specific responses. AZORIZ project aims at characterizing the molecular basis of the specificity of associative sy mbiosis between *Azospirillum lipoferum* strains andtheir corresponding host rice culti var. With a simplified and checked system of inoculation, the following analyses were conducted seven days after inoculation : (i) the plant growth-promoting effect of each strain on the two rice cultivars; (ii) the rice root colonization patterns; (iii) themetabolomic response of the host; (iv) the transcriptomic response of the plant; (v) the transcriptomic response of *Azospirillum.* The results obtained concerning the parameters of growth show a differential response of the plant according to the inoculated strain and indicate a stronger effect when a strain is tested on its cultivar of origin. Moreover, significant modifications of the secondary metabolism on both rice cultivars were shown in response to the inoculation, with higher effect for extracts from roots (by comparison to extracts from leaves).We provide the first results of the transcriptomic responses of both partners in order to identify the potential genetic determinants defining thespecificity of interacti on and to test at the wholegenome scale the genes/TE interactions in response to these biotic interactions.

Keyword : Transcriptome, Rice, *Azospirillum*, Transposable Element

PC-51

Preliminary proteomics analysis in young panicle of Thermo-sensitive genic male sterility rice lines

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Thermo-sensitive genic male sterility (TGMS) facilitates hybrid production. Hybrid rice has 15 to 20 percent yield advantage over the best inbred varieties. In this study, a proteomic approach was used to investigate proteins involved in TGMS using 3 TGMS rice lines.The three TGMS rice lines have different genetic backgrounds, and are controlled by different *tgms* genes. Total young panicle proteins under sterile (high temperature) and fertile (low temperature) conditions were extracted and separated using GeLC-MS/MS. A total of 803 proteins were detected. Using at least 30% difference in levels of expression at fertile and sterile conditions of each rice line, 18 proteins were shown to be up-regulated and 11 proteins were shown to be down-regulated, concordantly in all the three TGMS lines. These proteins are involved in several biological processes such as defense/stress responses, lipid metabolism, transcription, glycolysis and signal transduction. The information obtained from this study could be useful in rice breeding programs for hybrid production and for resistant lines to overcome temperature changes in the future.

Keyword : Proteomics, TGMS, GeLC-MS/MS, Hybrid, *Oryza sativa*

PD-01

Sequence variation of rice blast fungus, *Magnaporthe oryzae*, avirulence genes in Thailand

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The interaction between rice, *Oryza sativa*, and rice blast fungus, *Magnaporthe oryzae*, is triggered by an interaction between the protein products of the host resistant gene, and the pathogen avirulence gene. The resistant gene has been effectively protecting rice plant from rice blast infection. However, the resistant genes usually break down several years after the release of the resistant rice varieties because the fungus has evolved to new races. The objective of this study is to investigate the nucleotide sequence variation of avirulence genes including *AvrPita*, *AvrPik* and *AvrPii* that influence the adaptation of rice blast fungus to overcome the resistant genes. Sixty rice blast fungus isolates were collected in 2005 and 2010 from infected rice plants in northern and northeastern Thailand. The nucleotide sequences of avirulence genes were amplified and analyzed. The results showed high level of nucleotide sequence polymorphisms and the positive genetic selection pressure overtime in Thai blast isolates. Interestingly phylogenetic analysis indicated that Thai blast isolates were different from blast isolates from other part of the world. The details of sequence variation analysis were described. The information from this study can be used for rice blast resistant breeding program in the future.

Key words: nucleotide variation, rice blast disease, avirulence gene, rice blast fungus

PD-02

The XA21-associated kinase1 (*OsSERK2*) regulates immunity mediated by the XA21 and XA3 immune receptors

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The rice XA21 pattern-recognition receptor and the structurally related XA3 receptor, confer immunity to *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), the causal agent of bacterial leaf blight disease. Here we report the isolation of OsSERK2 (rice somatic embryogenesis receptor kinase 2), an ortholog of *Arabidopsis* BAK1 (brassinosteroid-insensitive 1 (BRI1) associated kinase 1) and demonstrate that OsSERK2 positively regulates immunity mediated by three pattern recognition receptors: XA21, XA3 and rice FLS2 (OsFLS2). Rice plants silenced for *OsSerk2* also display altered morphology and reduced sensitivity to the hormone brassinolide. In contrast to *Arabidopsis* BAK1, OsSERK2 undergoes bidirectional trans-phosphorylation with XA21 *in vitro* and forms a constitutive complex with XA21 *in vivo,* even in the absence of elicitor treatment.

Keyword : Pattern recognition receptor, *Xanthomonas*, disease resistance

PD-03

Potential resource for rice bacterial blight disease resistance in the newly bred lines of Taiwan

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Thirteen accessions, which showed resistance to bacterial blight *Xanthonomas oryzae* pv. oryzae (Xoo) at level of moderate resistance (MR) and resistance (R) over two years (2008-2009) at Taichung DARES nursery, were selected to characterize their resistance to the bacterial blight isolate XF89b collected in Taiwan by TARI. Inoculation of the XF89b isolate to these selected accessions showed the consistent results as the two-year trials at the Taichung DARES nursery. To address whether resistance to bacterial blight in these thirteen accessions attributed to known bacterial blight resistant genes, we further characterized the genotypes at the xa5, Xa7, xa13, or Xa21 loci in these thirteen accessions. The result showed that only genotypes of the Xa7 marker are polymorphic among different accessions: the Xa7 resistant allele was detected in TN1 (the susceptible control), CNSWY892220, MLS-Xa21, and MLS-Xa7, and the Xa7 susceptible allele was found in the rest of ten accessions. Because TN1 was used as the susceptible control in the field test, the disease resistance cannot be attributed to the Xa7 resistance. We then conducted additional experiment to identify the avirulent genes in the XF89b isolate. The results showed that IRBB4, IRBB5, IRBB7, and IRBB21 were resistant to the XF89b isolate. Therefore, we concluded that resistance to bacterial blight in these 13 accessions might result from the resistance of the Xa4 gene, other R genes which were not included in this set of the IRBB differential hosts, or genes conferring partial resistance. Keywords: bacterial blight (*Xanthonomas oryzae* pv. oryzae), rice (*Oryza sativa* L.), resistant gene, Taiwan

Keywords: bacterial blight (*Xanthonomas oryzae* pv. oryzae), rice (*Oryza sativa* L.), resistant gene, Taiwan

PD-04

Identifying a new source of a bacterial blight resistance gene *xa5* in rice variety 'IR62266' and development of a functional marker 'PA*xa5*', the easy agarose based detection

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Bacterial blight (BB) caused by *Xanthomonas oryzae pv. oryzae (Xoo)* is found worldwide and can cause tremendously damage to rice yield in many countries. The use of resistance rice varieties is the most economic and effective method to manage and control this disease. IR62266, a broad spectrum BB resistance and a promising rice line in Thailand which developed by IRRI, was characterized in this study. Three Thai isolates of *Xoo,* TXO1*,* TXO2*,* and TXO3 were employed to identify a source of BB resistance in IR62266. A total of 190 F_3 individuals were developed from crosses between IR62266 and KDML105, a susceptible variety to BB. The reactions to three races have shown that the F_3 population segregated in 3 susceptible: 1 resistant, thereby confirming that BB resistance in IR62266 was controlled by a single recessive gene. RM122 specifying on short arm of chromosome 5 was clearly identified homozygous resistant genotypes carrying alleles from IR62266 from susceptible genotypes in F_3 progeny. Multiple regression analysis displayed that RM122 was the tightly linked marker to a resistance gene*xa5* which accounted 66.03%*.* Later, PA*xa5,* a new functional SNP marker, has been developed on the basis of sequence of the cloned *xa5* gene to identify varieties carrying *xa5* resistance gene. This marker was validated in a wide range of germplasm and now can be implemented in marker-assisted breeding to facilitate selection for this broad spectrum BB resistance gene *xa5.*

Keyword : *xa5*, Bacterial blight, Rice, Functional marker

PD-05

A defense-responsive gene, *DR153*, negatively regulates rice disease resistance

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Previous results revealed that activation of rice *WRKY13* gene, encoding a WRKY-type transcription factor-like protein, enhances rice resistance to *Xanthomonas oryzae* pv*. oryzae* (*Xoo*), which causes bacterial blight disease, and *Magnaporthe oryzae*, which causes blast disease. Here we report that one rice gene *DR153*, also encoding a WRKY-type protein, is specially targeted by WRKY13 both *in vitro* and *vivo*. The expression of *DR153* was suppressed in *WRKY13*-overexpressing lines and induced in the *WRKY13*-suppressing lines with or without *Xoo* and *M. oryzae* infection. Overexpression of *DR153* increased rice susceptibility to *M. oryzae*, while suppression of *DR153* enhanced rice resistance to *M. oryzae*. However, the *DR153*-transgenic plants showed no obvious difference from wild-type plants to *Xoo* infection. These results suggested that *DR153* is a negatively regulator in rice response to *M. oryzae* and it appears to function downstream of *WRKY13* in rice defense transduction pathway.

Keyword : WRKY transcription factor

PD-06

Simultaneous quantification of multiple phytohormones and metabolites in rice–bacterium interaction

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We have developed the method for simultaneous quantification of multiple phytohormones, abscisic acid, indole-3-acetic acid (IAA), jasmonic acid (JA), and salicylic acid, and gibberellins (GA1, GA4, GA53), hormone conjugates, IAA-aspartic acid (IAA-Asp), JA-isoleucine, and methyl JA, and phytoalexins, momilactone A, naringenin, and sakuranetin using ultra fast liquid chromatography–electrospray ionization tandem mass spectrometry. Using this method, we analyzed the dynamic profiles of these compounds in the interactions of rice and bacterial pathogen.

Keyword : phytohormones, UFLC/MSMS

PD-07

Dissection of resistance to brown spot in multi-parent advanced generation inter-cross (MAGIC) and mutant populations

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Brown spot disease caused by *Bipolaris oryzae* is prevalent in south, south-east Asia and Africa. The disease is severe under cool and wet condition. Here we report two approaches to explore the genetic basis of resistance to *B. oryzae* using two unique populations - a highly recombined Multi-parent Advanced Generation Inter-Cross (MAGIC) *indica* population and an IR64 mutant population. A Genome-Wide Association (GWA) mapping approach identified a major QTL on chromosome 12 and minor QTLs on other chromosomes for resistance to brown spot (isolate SM2). The major QTL on chromosome 12 may account for 40-50% variation and in this region there are genes encoding proteins associated with disease resistance, e.g. RPS2 and RPM1. Further studies would validate the minor QTLs and develop trait specific SNP markers for marker aided breeding. In parallel, we screened for IR64 mutants with altered disease response to elucidate the disease resistance pathways. We have identified two mutants - D6766 exhibiting loss of resistance to blast and D10808 showing loss of resistance both blast and brown spot. Genetic analysis suggests that the loss of resistance in D6766 and D10808 was controlled by two independent recessive mutations. The mutation in D10808 appears to be important for resistance to two diseases. A double mutant population (D6766 x D10808) has been created to study the combined effect of the two mutations. Results from both approaches would enable a better understanding of resistance to brown spot and possibly identify genes conferring resistance to multiple diseases.

Keyword : Brown spot MAGIC IR64 mutants

PD-08

Plant innate immunity mediated by rice LysM receptors

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Plants have the ability to recognize microbe/pathogen-associated molecular patterns (MAMPs/ PAMPs) and initiate various defense responses. This defense system is strikingly similar to the innate immunity of animals. Chitin, a major component of fungal cell wall, is a representative fungal MAMP and triggers defense signaling in a wide range of plant species. Recently, we identified two types of chitin receptors, CEBiP and CERK1, which play important roles for chitin recognition and defense signaling in rice and *Arabidopsis*[1-3]. In rice, both CEBiP and OsCERK1 are required for chitin oligosaccharide-induced defense responses. CEBiP is a GPI anchored protein and has several LysM domains in the extracellular region, which relate its high chitin binding activity. Here we will discuss the relationship between the structure of those LysM domains and sugar binding specificity in CEBiP and also the formation of a receptor complex in the presence of chitin oligosaccharide.1) Kaku et al, *PNAS*, 103, 11086 ('06), 2) Miya et al, *PNAS*, 104, 19613 ('07) 3) Shimizu et al, *Plant J.,* 64, 204 ('10).

Keyword : Chitin, receptor, MAMP, LysM, immunity

PD-09

Diversity of cysteine-rich antimicrobial-like peptides in *Oryza sativa* complex species

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Defensive and reproductive protein genes seem to evolve more rapidly than other genes. Small, cysteine rich secreted peptides (CRPs) act as antimicrobial agents and function in plant intercellular signaling are over-represented among proteins expressed in reproductive tissues. As defensive and/or reproductive proteins, can *Oryza* CRPs be said to evolve rapidly? How diverse are CRPs in closely related *Oryza* species and what is the source of the variation? We surveyed the CRP gene sequences of six *Oryza* genomes comprising *Oryza sativa* ssp. japonica and ssp. indica, *Oryza glaberrima* and three accessions of *Oryza rufipogon*. The main cause of variation among *Oryza* CRPs appears to be gene loss or duplication. Among 541 CRP orthologue groups, 338 had at least one missing or non-viable member among the six genomes tested. 86 groups had additional members while 75 orthologue groups had both additional and missing or non-viable members. Polymorphisms in viable CRP gene coding sequences were relatively few and the coding sequences are short, making it difficult to measure mutation rates. However, 35 orthologue groups had a Ka/Ks ratio greater than 1.5 in at least one pairwise species combination. 6 of these groups represented defensin-like protein genes expressed in early seed development. *Oryza* genomes, like other plant genomes, seem to accumulate large reservoirs of CRP sequences. Some of these CRP sequences appear to be dispensable. Potentially, several CRP proteins acting together might contribute a phenotype. In that case, genome plasticity within CRP sequence containing regions may be a source of phenotypic variation.

Keyword : cysteine rich antimicrobial peptide

PD-10

Enhanced resistance to bacterial and fungal pathogens by overexpression of a human cathelicidin antimicrobial peptide (hCAP18/LL-37) in rice

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The human cathelicidin antimicrobial protein hCAP18, which includes the C-terminal peptide LL-37, is a multifunctional protein. As a possible approach to enhancing the resistance to plant disease, a DNA fragment coding for hCAP18/LL-37 was fused at the C-terminal end of the leader sequence of endopolygalacturonase-inhibiting protein under the control of the cauliflower mosaic virus 35S promoter region. The construct was then introduced into rice. LL-37 expression was confirmed in transgenic plants by reverse transcription-polymerase chain reaction and western blot analysis. Transgenic plants exhibited varying levels of resistance to bacterial and fungal pathogens. The average size of disease lesions in the transgenic plants was reduced to less than half of that in wild-type plants. Our results suggest that the antimicrobial LL-37 peptide is involved in wide-spectrum resistance to bacterial and fungal pathogen infection.

Keyword : Antimicrobial peptide, Cathelicidin, Disease resistance, Transgenic rice

PE-01

Expression of *OsHSP16.9* gene under salt, dehydration, heat and ABA treatment

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Plants have adapted the ability to respond to various abiotic stresses such as high salinity, osmotic stress, high and low temperatures, as well as drought in order to survive. Heat shock proteins (HSPs) are conserved in prokaryotes and eukaryotes and are especially abundant in plants. Also, HSPs are one of the main expressed products of the cell in response to stresses. HSPs can be divided into six families such as HSP100, HSP90, HSP70, HSP60, small heat shock proteins (sHSPs) and ubiquitin (8.5 kDa). A group of sHSPs are conserved as intra-cellular chaperones for other proteins in a molecular mass ranging from 15 to 42 kDa. The sHSPs are much more abundant in higher plants than in other organisms and the cytosolic class I small heat shock proteins represent the sHSPs in plants. The sequence similarity can be up to 93% and identity up to 85%. This was observed in several assays examining growth status over development, including increased germination, fresh weight, and length of shoots and roots as well as enhanced chlorophyll retention. These results suggest that the transcription factor *OsHSP16.9* is an important determinant of stress response in plants and that changes in its expression level in plant could increase stress tolerances.

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Keyword : abiotic stresses, small heat shock proteins, *OsHSP16.9*, transcription factor

PE-02

Partial characterization of rice class II E3 *Ubiquitin ligase OsPUB2* (*Oryza sativa* putative U-box 2), which is involved in abiotic stress responses

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Ubiquitin-mediated post-translational protein modification is commonly found in eukaryotes. The ubiquitin-26S proteasome pathway regulates various cellular and physiological processes, such as cell division, flowering time, biotic and abiotic stress responses. Rice contains at least 77 U-box motif-containing E3 ubiquitin ligases. These U-box E3 ligases are divided into 8 class depending on their domain organization. We focused class II U-box E3 Ub ligases, which are composed of a single U-box domain and ARM (Armadilo) repeat domain. To characterize whether class II U-box E3 ligases are involved in abiotic stress responses, we performed semi-quantitative RT-PCR under various abiotic stress treatments. We identified that expression of *OsPUB2* (*Oryza sativa putative U-box 2*) was dramatically induced by abiotic stress, including drought, salt, and cold stresses, but not by ABA. In contrast, *OsPUB3*, a homolog of *OsPUB2*, was constitutively expressed. Both bacterially expressed OsPUB2-MYC and OsPUB3-MYC had E3 ubiquitin ligase activities with *Arabidopsis* UBA1 (E1) and UBC8 (E2) *in vitro*. To determine the role of *OsPUB2* and *OsPUB3 in vivo, OsPUB2*- and *OsPUB3*-over-expressing transgenic plants as well as *ospub2* and *ospub3* single and double RNAi knock-down rice plants were generated. Genomic Southern blot analyses identified several independent transgenic lines. Their phenotypes in relation with abiotic stress responses will be presented.

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Keyword : ubiquitination, U-Box, abiotic stress

PE-03

Molecular characterization of rice LEA I gene - protein function and gene evolution

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Proteins abundant in seeds during the late stages of development, late embryogenesis abundant (LEA) proteins,are associated with desiccation tolerance. More than 100 of the group I *LEA* genes, also termed *Em* genes, have been identified from plants, bacteria, and animals. The wide distribution indicates the functional importance of these genes. Under *in vitro* condition, the biophysical features of rice Em and Em-like proteins indicated that the proteins were natively unfolded in solution status and underwent desiccation-induced conformational changes. Besides, the proteins interacted with non-reducing sugars and phospholipids after drying. However, unlike other hydrophilic LEA proteins, Em and Em-like proteins failed to affect the conformations of poly-L-lysine. Thus the function of Em and Em-like proteins might involve in the formation stability of bioglasses or plasma membrane. The same location of the sole intron indicated that plant *Em* and *Em*-like genes came from a common ancestor. However, rice *Em*-like locus locates at a 193-Kb segment in chromosome 1 and is conserved in several published cereal genomes. Thus, the ancestor of *Em*-like genes might have evolved before the divergence of Poaceae from other monocots.

Keyword : CD, Em-like, evolution, FTIR, LEA protein, rice

PE-04

Development of transgenic rice lines using FOX-hunting system and identification of genes with abiotic stress tolerance

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The latest report on draft genome of *Brassica rapa* sequence has been published. To elucidate the functions of a large population of these genes and to search efficiently for agriculturally useful genes, the Full-length cDNA Over-expressor (FOX) gene hunting system was used. The FOX library was transformed into rice by *Agrobacterium*-mediated transformation. Approximately 1,150 FOX-rice lines were generated. Genomic PCR analysis indicated that the average length of FL-cDNAs was 900~1,200 bp with functional annotation of many unknown function (35.5%). Most of the randomly selected transgenic rice lines showed overexpression (92%) and barely mRNA expression in the wild type Gopum. Moreover, 94% of the 850 transgenic rice lines were moderately tolerant (slightly yellow) to cold and 9 lines were tolerant (seedling light green). For the salinity evaluation, most of the transgenic lines (85%) were highly susceptible whereas seven lines were tolerant. In addition, morphological evaluation of rice lines showed minimal phenotypic alteration (12%). About 25.1 and 22% were earlier in terms of days to heading and chlorophyll contents, respectively. Further, 18% of FOX rice lines showed lower chlorophyll contents. Filled grains, number of tillers, panicle length, culm length and plant height were relatively less variable among the lines. These results provided useful genes for functional analyses in the mechanisms of identified transgenic tolerant lines.

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Keyword : FOX, abiotic stresses, functional analysis, *Brassica rapa*, rice

PE-05

A modified rice αAmy8 promoter significantly enhances hypoxia-inducible expression of a human epidermal growth factor in transgenic rice seedlings

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Expression of α-amylase genes in rice is regulated repressively by sugar in germinated embryos and suspension cells and inductively by gibberellin (GA) in germinated endosperms through the sugar response complex (SRC) and the GA response complex (GARC), respectively. Promoters of two rice α-amylase genes, *aAmy3* containing only SRC and *aAmy8* containing both SRC and GARC, have been shown to direct high-level production of recombinant proteins in rice cell cultures and germinated seeds. The *aAmy8* gene is highly expressed in the endosperm of seeds during germination, whereas the promoter activity of *aAmy3* is stronger than that of *aAmy8* in cultured rice suspension cells, protoplasts, and anoxic coleoptiles. In the present study, deletion analysis demonstrates the *aAmy3* SRC confers hypoxia responsiveness to a minimal promoter in transgenic rice, and was designated as *aAmy3* SRC/HRC (sugar and hypoxia response complex). Loss-of-function assay show that the G box and duplicated TA box are necessary for hypoxia induction and expression of *aAmy3* SRC/HRC. To enhance *aAmy8*promoter activity, we modified the *cis*-acting DNA elements within the SRC/GARC of *αAmy8* promoter. We found that the addition of the G box and duplicated TA box leads to high-level expression of*aAmy8* SRC/GARC and significantly enhances *aAmy8* promoter activity in transformed cell cultures and germinated transgenic rice seeds. We also show that these modifications have drastically increased the activity of *aAmy8* promoter in rice seedlings during germination under hypoxia. The modified *aAmy8* promoter was used to produce the recombinant human epidermal growth factor (hEGF) in transformed rice cells and hypoxic transgenic rice seedlings. We found that the recombinant hEGF proteins are stably produced with yields comprising up to 1.8% of total soluble protein (TSP) in the transformed cells and remain biologically active. The expression level of synthetic hEGF containing preferred rice codon usage comprises up to 7.8% of TSP in hypoxic transgenic seedlings. Our studies reveal that the modified *aAmy8* promoter will be very useful in establishing a novel expression system for the high-level production of foreign proteins in transformed rice suspension cells and transgenic rice seedlings under hypoxia.

Keyword : rice, α-amylase promoter, promoter modification, hypoxia, EGF

PE-06

Cloning and characterization of a stress-inducible small GTPase gene from jasmine rice

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Small GTP-binding proteins from the Rab family are involved in intracellular trafficking and play a critical role in several plant development processes. A gene coding small-GTP binding protein (*OsRab5*) was cloned from jasmine rice (*Oryza sativa* L. cv. KDML105). The deduced amino acid sequence of the gene exhibited 98% identity with *Oryza sativa* (japonica cultivar group). All the characteristic motifs of small GTP-binding Rab family protein were found in OsRab5 as well. The expression analysis revealed that this gene was monitored in all rice tissues such as roots, young leaves, senescing leaves, flowers and seeds. The expression of *OsRab5* in stress conditions was performed. Interestingly, expression levels of *OsRab5* increased by a factor of 2 to 6 on leaves incubated in salinity, abscisic acid (ABA), ethylene and dark treatment and increased slightly on wounded leaves compared with control leaves. Hence it is suggested that *OsRab5* act as a stress-inducible gene.
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Keyword : small GTP-binding protein, *OsRab5* expression profile, stress-inducible gene

PE-07

Constitutive activation of transcription factor OsbZIP46 improves drought tolerance in rice

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OsbZIP46 is one member of the third subfamily of bZIP transcription factors in rice (*Oryza sativa*). It has high sequence similarity to ABA-responsive element binding factor (ABF/AREB) transcription factors ABI5 and OsbZIP23, two transcriptional activators positively regulating stress tolerance in Arabidopsis (*Arabidopsis thaliana*) and rice, respectively. Expression of *OsbZIP46* was strongly induced by drought, heat, hydrogen peroxide, and abscisic acid (ABA) treatment; however, it was not induced by salt and cold stresses. Overexpression of the native*OsbZIP46* gene increased ABA sensitivity but had no positive effect on drought resistance. The activation domain of OsbZIP46 was defined by a series of deletions, and a region (domain D) was identified as having a negative effect on the activation. We produced a constitutive active form of OsbZIP46 (OsbZIP46CA1) with a deletion of domain D. Overexpression of *OsbZIP46CA1* in rice significantly increased tolerance to drought and osmotic stresses. Gene chip analysis of the two overexpressors (native *OsbZIP46* and the constitutive active form *OsbZIP46CA1*) revealed that a large number of stress-related genes, many of them predicted to be downstream genes of ABF/ AREBs, were activated in the *OsbZIP46CA1*overexpressor but not (even down-regulated) in the *OsbZIP46* overexpressor. OsbZIP46 can interact with homologs of SnRK2 protein kinases that phosphorylate ABFs in Arabidopsis. These results suggest that OsbZIP46 is a positive regulator of ABA signaling and drought stress tolerance of rice depending on its activation. The stress-related genes activated by OsbZIP46CA1 are largely different from those activated by the other rice ABF/AREB homologs (such as OsbZIP23), further implying the value of OsbZIP46CA1 in genetic engineering of drought tolerance.

Keyword : rice; bZIP, drought; constitutive active, phosphorylation

PE-08

Mapping traits associated with prolonged drought and heat tolerance in Nagina 22 mutant

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Drought and heat limit rice productivity considerably. N22 is a deep-rooted, drought and heat tolerant aus rice cultivar. Earlier, a dark green leaf mutant NH-*dgl 219* was isolated under prolonged drought and heat (Biologia Plantarum: 55(4), 721-724, 2011). In 2-D gel electrophoresis of leaf samples of N22 and mutant, a large spot in the mutant identified as Ribulose bisphosphate carboxylase large chain precursor (EC 4.1.1.39) distinguished it from N22. Mutant was further compared with N22 for several traits in field grown plants under normal and heat stress conditions (~50°Cday / ~30°C night) during dry season 2011. The temperature was raised just before booting and upto maturity by covering polythene sheets over an artificial tunnel on one set plants. The decrease in trait values of N22 and *dgl219* respectively were 24.9% and 20.9% in tiller number, 43.1% and 31.8% in panicle number, 20.5% and 2.1% in total amount of chlorophyll, 23.1% and 15.8% in electron transport rate 5.5% and 1.8% in Fv/ Fm and 5.5% and 2.5% in pollen number. However, the relative water content increased to 1.4% and 4.4%. Mapping of mutation in IR64×*dgl219* population was carried out for SPAD chlorophyll meter value in 36 F2 segregants with extreme phenotype. Marker locus RM229 on chromosome 11 was linked to SPAD value and 94% alleles were like *dgl219* allele. Mapping using 73 F2 segregants with extreme phenotype for 5 phenotypic traits showed RM 584 on chromosome 6 was linked to height, tiller number, leaf thickness and senescence and 71% alleles were from IR64.

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Keyword : heat, photosynthesis, N22, mutant, markers

PE-09

Detection of *cis*-acting regulation on drought-response genes in rice by allele specific expression imbalance

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Phenotypic variation has been attributed mostly to changes in the coding regions of genes. However, a growing number of studies show that the non-coding regions of genes have also contributed to the phenotypic expression of an organism. This study was undertaken to investigate if cis-acting regulatory elements play a role in drought response by examining allele-specific expression of the F1s. Two genetically similar backcross introgression lines (BILs), IR77298- 5-6-B-18 (drought tolerant) and IR77298-5-6-B-11 (intolerant) were crossed to produce F1 to identify the genes showing allelic imbalance (AI) by RNA sequencing. Two QTL regions conferring yield under drought stress on chromosomes 9 and 10 were detected in 5-6-B-18 by genetic mapping. Within the chromosome 9 and chromosome 10 QTL regions, we found 33 and 8 genes exhibiting AI, respectively. These genes co-localized with previously detected drought response QTLs. Of the 41 genes within the QTL regions, there was no significant difference in the degree of imbalance observed under normal and moderate drought stress conditions. Based on gene ontology, 9 of the 41 genes were considered putative drought-response genes. Using the IR64 genome assembly, the corresponding upstream regions of the genes exhibiting AI were analysed. Results revealed 23 different cis-acting elements that could influence transcript abundance. Some of these regulatory elements are known to be involved in dehydration response, defence signalling and carbohydrate breakdown. Results suggest that allelic imbalance mediated by cis-acting elements may play a role in the expression of drought-response genes.

Keyword : cis-acting regulation, allelic imbalance, drought

PE-10

Identification of a stress-inducible protein phosphatase gene SPP1 mediating drought resistance through reactive oxygen species scavenging by ABA-independent manner in rice

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Plants are exposed to diverse abiotic stresses including drought and salt throughout their life cycles. Plants response to abiotic stresses at molecular levels by perception and transduction of stress signals through a complexity of signaling pathways. Reversible protein phosphorylation mediated by protein kinases and protein phosphatases is a major event in signal transduction. Many protein phosphatase genes in rice were found to be induced by drought stress in diverse rice varieties by whole genome expression analysis. One of them designated as SPP1 (Stress-induced Protein Phosphatase 1) encoding a protein phosphatases 2C, was further characterized. The spp1 mutant was more sensitive than wild-type plants to drought stress at both seedling and panicle development stages. Plants transformed with an SPP1 artificial microRNA construct were also hypersensitive to drought stress. Microarray analysis of the mutant revealed that genes encoding reactive oxygen species (ROS) scavenging enzymes (glutathione S-transferase) were down-regulated in spp1 mutant. The spp1 mutant increased sensitivity to oxidative stress trigger by methyl viologen result from reduced activity of ROS-scavenging enzymes. Overexpression of SPP1 enhanced osmotic and oxidative stress tolerance. Expression of SPP1 was induced by drought, cold, heat shock and UV treatment, but not induced by abscisic acid. SPP1 was not interacted with SAPK2 and the ABA sensitivity of spp1 mutant and SPP1-overexpressing plants was not altered. Together, these results suggest that SPP1 might be a novel PP2C gene that medicated drought and oxidative stress by regulating ROS homeostasis through ABA-independent pathway.

Keyword : PP2C, drought, ROS, ABA-independent

PE-11

An OsbZIP23-interacting protein, BIP4, negatively regulating of ABA signaling and drought stress response in rice

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Plants have evolved complicated protective mechanisms to survive the adverse environmental condition. Hereinto, phytohormone abscisic acid (ABA) plays pivotal roles in the abiotic stress tolerance of plants. Previously, we identified that OsbZIP23 acted as a positive regulator of ABA signaling to regulate drought stress resistance of rice by modulating many stress-related genes (Xiang et al., 2008). Here, we identified a novel OsbZIP23-interacting protein, designated as BIP4, by yeast two-hybrid screening. BIP4 was co-localized in the nucleus and interacted with OsbZIP23 *in vivo*. The expression of *BIP4* was induced by ABA and drought stress but the induction was much less and slower than that of *OsbZIP23*. Overexpression of *BIP4* in rice resulted in decreased ABA sensitivity and drought tolerance, whereas two allelic *bip4* mutants (*bip4-1*and *bip4-2*) showed increased ABA sensitivity and drought tolerance. Moreover, the expression levels of OsbZIP23 target genes, including *RAB21*and *OsPP2C* that are positively regulated by OsbZIP23, were inversely correlated to the expression level of *BIP4*. Sequence analysis indicated that BIP4 is function-unknown protein with significant homologs existing in other plants. Our data together suggest that BIP4 acts as a partner of OsbZIP23 to negatively regulate ABA signaling and drought stress response in rice.
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Keyword : rice; OsbZIP23-interacting protein; ABA signaling; drought stress

PE-12

Molecular and biochemical screening and evaluation of transgenic drought tolerant rice

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Transgenic technology offers new prospects to increase rice yield under drought by modulating expression of genes involved in drought response metabolic pathways thereby increasing its tolerance to drought and other abiotic stresses. At the International Rice Research Institute (IRRI) Genetic Transformation Laboratory, a series of genetic constructs harboring candidate genes for drought tolerance under constitutive and drought inducible promoters were introduced into IR64, a popular high yielding variety. Approximately 100-120 independent transformants were developed per constructs and screened for better yield under drought. Prior to screening, each event was genotyped and molecularly characterized. Gene integration and number of copies were first determined by PCR and Southern analysis. Hygromycin leaf painting and petri plate growing assays were developed to increase the efficiency of the genotyping and homozygosity determination based on Mendelian segregation. Single copy, fertile T1 events were then selected and subjected to various drought environments in rain-out screen houses and contained field trials. To elucidate the correlation between the gene expression, phenotypic characteristic and certain physiology mechanisms, we conducted real time and semi quantitative PCRs to quantify the transgene expression levels and abscissic acid (ABA) content, osmolite, carbohydrate content analyses using ELISA and colorimetric techniques. The flow and protocols of these biochemical and molecular approaches are presented.

Keyword : transgenic, hygromycin, real time PCR, semi quantitative PCR, ABA, osmolites

PE-13

A rice kinesin gene is probably involved in drought stress

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Rice is one of the most important cereal crops and is a model for grain plants. Drought is an important abiotic stress limiting rice growth and productivity. Under drought condition, some rice cultivars having gene expression changes leading to tolerance while in other instances the plants fails to adapt to drought stress resulting in yield reduction or dead of the plants. Using rice genes previously reported to be involved in drought stress from several transcriptomes, we study expression of 50 selected genes in two drought tolerant and two drought sensitive lines at reproductive stage under drought compared to well water conditions. We found that a gene encoding for kinesin motor domain containing protein was up-regulated in both drought tolerant lines, while it was down-regulated in both drought sensitive lines, suggesting that this gene is probably involved in drought stress. In both drought tolerant lines, this gene was predominantly expressed in panicles, but in drought sensitive lines it showed expression mainly in root or stem. Application of ABA under drought condition caused down regulation of this gene in both drought tolerant lines, opposite with that in both drought sensitive lines. DNA sequences of this gene in the 4 tested rice lines will be discussed.

Keyword : drought stress, kinesin, rice, ABA, gene expression

PE-14

Quantifying the contribution of aquaporins to modulation of rice root hydraulic conductivity under drought

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Because of its adaptation to flooded soil, lowland rice has a unique physiological response to drought, but little is known about the root structural and functional characteristics for water uptake under this stress condition. Aquaporins (AQPs) are water channels that can significantly decrease the resistance of the cell-to-cell water pathway in roots, thus providing a molecular basis to explore the adjustment of root hydraulic conductivity (Lpr) in response to environmental stimuli. Pressure chamber measurements indicated that, compared with well-watered conditions, Lpr was reduced by around 40% under drought in two lowland rice varieties contrasting in drought response, IR64 and Dular. To evaluate the contribution of AQPs to these responses, we measured the expression levels of AQPs as well as the Lpr of roots grown in soil under well-watered and drought stress conditions in the presence or absence of aquaporin inhibitors (salt and azide). Rice root AQPs were highly expressed and were transcriptionally regulated by diurnal variations in both well-watered and drought stress conditions. AQP inhibitors applied directly into the soil showed varied responses. Whereas salt application in soil did not result in a reduction of Lpr, application of azide into the soil for 30 minutes induced a strong reduction of Lpr under well-watered conditions (78% in IR64 and 60% in Dular). Under drought stress, azide application induced a lower inhibition of Lpr (35% in IR64 and 40% in Dular). Altogether, these results indicate that AQPs contribute to Lpr of IR64 and Dular and suggest that changes in AQP expression and activity may allow rice roots to adjust water flux under drought.

Keyword : rice, drought, root hydraulic conductivity, aquaporins

PE-15

Genome analysis of salt-tolerant rice

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Stress environmental conditions such as drought and saline soils can reduce a variety of activities essential for respiration and photosynthesis resulting in growth and yield reduction in NaCl sensitive plants. Plant tissue culture is one of the techniques that are widely used to improve crop quality. Somaclonal variation is the spontaneous mutation that has been observed in *in vitro* culture. Because it increases genetic variation among propagated clones, the significant application of this technique is used in the improvement program. The molecular and cytogenetic study had been reported that tissue culture-induce variation affected cytological abnormality, chromosome structure, nucleotide mutation, gene silencing and also induced the expression of genes. LPT123-TC171 is the salt and drought tolerant rice line which was selected from somaclonal variation of the original Thai rice, LPT123. Genome change was studied in 4 levels which are chromosome level (polyploidy, aneuploidy), chromosome structure, transposable element and DNA sequence change by whole genome sequencing analysis. Our study showed that LPT123-TC171 did not show the change in the set of chromosome (polyploidy) or number of chromosome (aneuploidy). Interestingly, in the study of chromosome structure, we found the significant deficiency of chromosome ends (telomere) in the mutant rice line. This change could result from the oxidative stress in the screening process.

Keyword : somaclonal variation, salt tolerance, telomere deficiency

PE-16

Loss of chloroplastic *glutathione reductase 3* enhancing susceptibility to salinity is caused by reduced glutathione redox state and retrograde signals from chloroplast to nucleus

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Glutathione reductase (GR) catalyzes the reduction of oxidized glutathione to reduced glutathione (GSH), an important antioxidant in plants. However, knowledge of GR activity in salt tolerance is limited. In this study, we functionally characterized rice *glutathione reductase 3* (*GR3*). Heterologous expression of GR3 in *E. coli* confirmed that it can translate protein with GR activity. *GR3* was primarily expressed in rice roots and was specifically induced by salt stress in seedlings. The GR3–GFP protein fusion was targeted to the chloroplast. In contrast to the wild-type, the *gr3* mutant was susceptible to salt and methyl viologen and could be complemented by transformation of a wild-type *GR3* gene. With salt stress, GR3 knockout reduced total GR activity by 18%, inhibited growth and maximal efficiency of photosystem II, decreased the ratio of GSH to oxidized glutathione and rapidly increased the level of H_2O_2 in leaf tissue. In response to salinity, *gr3* showed reduced mRNA expression of genes crucial for salt stress tolerance, including *phospholipase Dα1, calcium-dependent protein kinase 7, calcineurin B-like protein-interacting protein kinase 15, receptor-like kinase 1*, 2 zinc finger transcription factors, and *Na^+/H^+ antiporter* and *salt overly sensitive 1* transporters. Thus, loss of GR3 causes rice sensitivity to salinity and may contribute to an imbalanced antioxidative defense system, as well as decreased expression of salt-stress-tolerance genes; the process may be regulated by a retrograde signal from the chloroplast to nucleus.

Keyword : *glutathione reductase*; Rice; salinity; hydrogen peroxide; ascorbate- glutathione cycle

PE-17

OsMLD encoding MYB-like DNA binding domain increases tolerance to salt stress in rice (*Oryza sativa* L.)

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MYB-like domain (*MLD*) gene is a transcription factor that plays a diverse role in plant development and in response to abiotic stresses. In this study, we isolated and developed *CaMV35S::OsMLD* rice lines and determined its expression pattern under abiotic stresses. It has Myb_CC_LHEQLE superfamily similar to most transcription factor genes but with a very unique binding domain of SHLQKYR in the C-terminal region. Overexpressing rice lines showed enhanced tolerance to salinity with elevated mRNA transcript. Additionally, mRNA transcripts were up-regulated by ABA, H_2O_2 and dehydration stresses. Further investigation in the enhanced tolerance to salinity showed an increased accumulation of proline and a decreased in malondialdehyde contents indicating that *OsMLD* gene may be involved in the regulation of proline and osmolytes during abiotic stresses. These results showed that *OsMLD* gene could be used in the development of rice intended for soil with salinity-related problem.

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Keyword : *OsMLD*, salt tolerance, proline, transgenic, rice

PE-18

Salinity tolerance in rice (*Oryza sativa* L.) during germination

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Sea level rise due to global warming is a serious problem around the world. Because sea-level rise may make cultivation areas more saline, it is required to improve salt tolerance in crops. Rice is one of the major staple crops and provides food for one-half of the world population. Therefore, it is important to improve salt tolerance in rice. There are many studies about the salt tolerance of rice plants at a seedling stage, and Nona Bokra and Pokkali were reported as high salt tolerance varieties. Several studies about the germination failure of rice seeds due to high salinity have been reported. However, there are few reports on salt tolerance among rice varieties during germination. In this study, the varietal differences in salt tolerance for the germination of five varieties, Nona Bokra, Pokkali, IR24, IR29 and Nipponbare, were tested on MS medium saturated with the NaCl concentrations of 0, 0.5, 1.0, 1.5 and 2.0% on mass basis. Germination rates on four days after sowing exceeded 71% for the NaCl concentrations of 0 and 0.5% in all varieties. Furthermore, Nipponbare, a salt sensitive variety, had highest germination rates in all of NaCl concentrations and varieties used. We concluded that the stage of young seedling was more sensitive to salinity than that of germination. This observation was consistent with the conclusions previously reported. Moreover, the salinity effects on germination are known to damages to albumen. Therefore, further studies are needed for the salt tolerance of albumen at germination.

Keyword : Germination test, Salinity tolerance, NaCl

PE-19

Expression of sodium transporter *SKC1* in rice seedling relates to different tolerance response under hydroponic and soil culture salinity stress conditions

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Rice SKC1 is associated with K+ homeostasis in shoot seedling of salt-tolerant rice and mapped on the short arm of chomosome1 and is a member of HKT-type transporters expressed in the parenchyma cells surrounding the xylem vessels by unloading the excess Na+ at xylem from root to shoot. This protein is a Na+-selective transporter affecting the SKC1 involved in regulating Na+/K+ under salt stress expressed as salinity tolerance ability. In this study, a tolerant line FL530 (FL) was crossed with KDML105 (KD) to generate BC2F8 backcross isogenic lines (BILs) using markers governing SKC1. BILs and parents were stressed at 150 mM salt under hydroponic (HC) and soil culture (SC) conditions. Lines carrying FL-SKC1 and KD-SKC1 alleles were selected and those carrying FL-SKC1 had low Na+/K+ under HC but the opposite was observed in SC while parents and KD-SKC1 BILs had the same ratio under both conditions. To investigate the pattern of response between the parents at SKC1, RT-PCR was performed using root and shoot tissues collected at 0h, 2h, 4h,6h, 24h, 48h, 5d, and 10d after treatment. FL-SCK1 was expressed in both tissues from HC but was highly expressed in the root than in the shoot. Likewise, KD-SKC1 was also expressed in HC but comparatively less than FL in both tissues. Expression of SKC1 in FL and KD in SC was slow (beginning at 6 hr) and less compared with HC and was detected abundant in FL. In the shoot, the opposite was observed and FL-SKC1 was stable but relatively less than KD. Results indicate that SKC1 is functioning but is differentially controlled in both conditions for both parents. Expression analysis using selected lines above may provide answer to the differential control of SKC1 in different environments.

Keyword : salinity tolerance, SKC1, hydroponic, soil culture, Na+/K+

PE-20

Vacuolar Na+/H+ antiporter overexpression for salt tolerance: A complex regulation in rice

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The vacuolar Na^+/H^+ antiporter has been shown to alleviate saline stress by sequestering Na^+ in both WT Arabidopsis and rice and when overexpressed in many transgenic crops. The level of salt tolerance conferred in transgenics has been substantial in dicots like tomato and Brassica but only moderate in cereals like wheat and rice. Overexpression of the Nipponbare Na^+/H^+ antiporter 1.9 kb cDNA (5' UTR truncated at the 5' end and without the 460 bp 3' UTR) in the rice landrace Binnatoa (BA), conferred moderate salt tolerance which correlated well with the transcript levels at the seedling stage. Transformation of rice was with the cDNA corresponding to OsNHX1 transcript-2 (2394 bp), but not with transcript-1 (2265 bp) or transcript-3 (1820 bp). Transfer of the transgene into the high yielding farmer-popular background genome of BRRI dhan28 by cross-breeding, however lowered the level of tolerance originally obtained, despite production of comparable levels of the NHX1 protein in Western blots. In another transformation event cloning of the full cDNA of transcript-2 including full 5´ UTR, 3´UTR and coding region in BA showed better tolerance to salt stress emphasizing the importance of the 3´UTR in salt tolerance. The higher level of tolerance found in the control Pokkali, wild type BA and transgenic BA could be correlated with the levels of transcript-3 having a truncated 3´ UTR sequence, which was absent in WT and transgenic BRRI dhan28. Transcript-3 lacks the nucleotides coding for the C-terminal regulatory domain responsible for increasing Na^+/H^+ antiport activity and increasing Na^+ selectivity.

Keyword : NHX antiporter, salt stress

PE-21

Expression level of NHX1 gene, a vacuolar Na+/H+ exchanger, Na+ accumulation and physiological changes in rice (*Oryza sativa* L. ssp. *indica*) responses to salt stress

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Salt affected soil is one of the most abiotic stresses to inhibit plant growth and development as well as productivity, especially rice crop. Ion homeostasis is a candidate defense mechanism in salt tolerant plants or halophyte species to storage the salt toxic ions in the vacuoles. The aim of this investigation was to determine the *NHX1* (a vacuolar Na^+/H^+ exchanger) regulation by salt stress (200 mM NaCl) in both Pokkali salt tolerance and IR29 salt susceptible, the accumulation of Na^+ in the root and leaf tissues using Corona green straining assay and physiological changes. An expression of *NHX1* gene in the leaf tissues was evidently increased in salt stressed Pokkali, whereas that in salt stressed IR29 was unchanged. Na^+ in the root tissues of both Pokkali and IR29 was enriched when subjected to 200 mM NaCl for 12 h and then easy to detect in the leaf tissues of salt stressed plants for 24 h, especially in the Pokkali cultivar. Also, the activity of *NHX1* protein in the leaf tissues of Pokkali may regulate on Na^+ pumping into vacuole, leading to maintain the photosynthetic abilities. Maximum quantum yield (F_v/F_m), photon yield of PSII (F_{PSII}) and net photosynthetic rate (P_n) in salt stressed Pokkali leaves were better than those in salt stressed IR29, resulting in an enhancer amount of growth performance.

Keyword : Corona green straining, growth, ion homeostasis, photosynthetic abilities, sodium ion pumping

PE-22

Expression analysis and characterization of rice oligopeptide transport gene (*OsOPT10*) that contributes to salt stress tolerance

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Knock-out of a gene by insertional mutagenesis is a direct way to address its function through the mutant phenotype. Among ca. 15,000 gene-trapped *Ds* insertion lines of rice, we identified one line from selected sensitive lines in highly salt stress. We conducted gene tagging by TAIL-PCR, and DNA gel blot analysis from salt sensitive mutant. A gene encoding an oligopeptide transporter (OPT family) homologue was disrupted by the insertion of a *Ds* transposon into the *OsOPT10* gene that was located shot arm of chromosome 8. The *OsOPT10* gene (NP_ 001062118.) has 6 exons and encodes a protein (752 aa) containing the OPT family domain. RT-PCR analysis showed that the expression of *OsOPT10* gene was rapidly and strongly induced by stresses such as high-salinity (250 mM), osmotic, drought, 100 μM ABA. The subcellular localization assay indicated that *OsOPT10* was localized specifically in the plasma membrane. Overexpression of *OsOPT10* in *Arabidopsis thaliana* and rice conferred tolerance of transgenic plants to salt stress. Further we found expression levels of some stress related genes were inhibited in *OsOPT10* transgenic plants. These results suggested that *OsOPT10* might play crucial but differential roles in plant responses to various abiotic stresses.

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Keyword : Knock-out, insertional mutagenesis, *OsOPT10* gene, abiotic stresses

PE-23

Transcriptional regulations of the genes of starch metabolism and physiological changes in response to salt stress rice (*Oryza sativa* L.) seedlings

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The aim of this investigation was to compare the transcriptional expression of starch metabolism, involving genes and physiological characters, in seedlings of two contrasting salt-tolerant rice genotypes, in response to salt-stress. The soluble sugar content in rice seedlings of both salt-tolerant and salt-sensitive genotypes was enriched, relating to starch degradation, in plants subjected to 200 mM NaCl. In the salt-tolerant cultivar Pokkali, a major source of carbon may be that derived from the photosynthetic system and starch degradation. In starch degradation, only *Pho* and *PWD* genes in Pokkali were upregulated in plants subjected to salt stress. In contrast, the photosynthetic abilities of IR29 salt-susceptible cultivar dropped significantly, relating to growth reduction. The major source of carbohydrate in salt-stressed seedlings of the IR29 cultivar may be starch metabolism, regulated by *ADP-glucose pyrophosphorylase* (*AGP*), *starch synthase* (*SS*), *starch branching enzyme* (*SBE*), *starch debranching enzyme* (*ISA*), *glucan-water dikinase* (*GWD*), *dispropotionating enzyme* (*DPE*), *phospho glucan-water dikinase* (*PWD*) and*starch phosphorylase* (*Pho*). Also, the major route of soluble sugar in salt-stressed IR29 seedlings was that from starch metabolism, whereas in salt-stressed Pokkali seedlings it was from photosynthesis and starch degradation. This was identified as novel information in the present study.

Keyword : gene expression, *indica* rice, net photosynthetic rate, salt tolerance, soluble starch

PE-24

Identification of salt responsive genes in an advanced backcross population from a cross between *Oryza sativa* L. cv. Milyang23 and O. glaberrima

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Salinity tolerance has been extensively studied in *Oryza sativa*, but little is known about the salt tolerance levels in *O. glaberrima* and their inter-specific progenies. To explore beneficial genes for salinity tolerance, Milyang23 and *O. glaberrima* were evaluated under the salinity stress condition. 5 days after treatment with 100 mM NaCl stress, Significant differences between Milyage23 and *O. glaberrima* were detected in all traits related to salinity tolerance. These results seem to indicate beneficial effect of the *O. glaberrima* alleles for salt tolerance. qRT-PCR analysis was used to determine the expression profile of four known genes (SKC1, OsTPP1, OsMPK3, OsCHK11) related to salinity tolerance. Among the four genes, OsCHX11 was clearly up-regulated in the root of *O. glaberrima*, which continued up to 24h after the stress, when the transcript displayed the highest expression in root with induction approximately twelve times higher than the control. Expression pattern of OsCHX11 using introgression lines will be performed to check whether these genes are effectively expressed in Milyang23 genetic background.

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Keyword : Rice, Salinity, OsCHX11

PE-25

QTL mapping and confirmation for tolerance of anaerobic conditions during germination derived from the rice landrace Ma-Zhan (Red)

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The practice of direct seeding in rice has been adopted increasingly by farmers as it requires less labor and cost than conventional transplanting. However, flood-prone and uneven fields or heavy rainfall shortly after sowing on direct-seeded fields could lead to poor crop establishment as most rice varieties are susceptible to flooding during germination. Hence, tolerance of anaerobic conditions during germination is an essential trait to sustain rice production in these ecosystems. A quantitative trait locus (QTL) study was conducted to reveal the genetic basis of the tolerance of anaerobic conditions during germination using a population derived from a cross between a susceptible variety, IR42, and a tolerant landrace from China, Ma-Zhan (Red). Phenotypic data was collected based on the survival rates of the seedlings at 21 days after sowing of dry seeds under 10 cm of water. QTL analysis of the mapping population, which consists of 175 $F_{2:3}$ families genotyped with 118 simple sequence repeat (SSR) markers, identified six significant QTLs on chromosomes 2, 5, 6, and 7; in all cases, the tolerant alleles were contributed by Ma-Zhan (Red). The largest QTL on chromosome 7, having an LOD score of 14.5 and an R^2 of 31.7%, was confirmed using a BC_2F_3 population. The QTLs detected in this study provide promising targets for further genetic characterization and use in marker-assisted selection to rapidly develop varieties with improved tolerance of anaerobic conditions during germination. Furthermore, this trait can be combined with other abiotic stress-tolerance QTLs to provide resilient varieties for direct-seeded systems.

Keyword : submergence, anaerobic germination, direct-seeded rice, quantitative trait locus (QTL), Ma-Zhan (Red)

PE-26

Characterization of a major QTL for tolerance to anaerobic germination for direct-seeded systems

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Unprecedented population growth, urbanization and declining areas for agriculture along with climate change pose serious challenges for future food security, bringing about a high demand for intensification of rice cultivation. A shift from conventional transplanting to direct seeding is seen as beneficial as it can ease the way into mechanization and reduce the cost of production, enable more efficient water use and improve crop rotation. Considerable shortcomings in direct-seeded systems, namely weed invasion and failure of seedling establishment in areas that experience frequent flooding or in unleveled fields, have prevented its widespread adoption. Tolerance to anaerobic germination (AG) enables plants to cope with early flooding and thus is an essential trait for direct-seeded varieties. However, most rice varieties are unable to survive anaerobic conditions during germination and so far only few landraces with high tolerance of AG stress are known. Phenotyping of a mapping population between the AG-tolerant Myanmar landrace Khao Hlan On (KHO) and the AG-susceptible mega-variety IR64 led to the identification of a major QTL for AG (*AG1*) on chromosome 9. Development of BC_4F_4 near isogenic lines (NIL) allowed for fine-mapping of *AG1* to a ~58 kb region containing five putative candidate genes (*AG1-A* to *AG1-E*). Evaluation of KHO, NIL and IR64 lines germinated under submergence suggests that *AG1* confers high AG tolerance through enhanced coleoptile elongation. Correlation of amylase activity with coleoptile length suggests efficient starch mobilization to be responsible for sustained growth. Expression studies point to *AG1-D* as the gene underlying *AG1*.

Keyword : direct-seeding, anaerobic germination, AG1, Khao Hlan On

PE-27

Gene Validation of a Major QTL for Tolerance of Anaerobic Conditions during Germination

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Most rice varieties cannot survive prolonged submergence during germination and early seedling establishment. However, several landraces that display a pronounced tolerance to flooding during germination were identified. This unique ability has been associated with efficient starch mobilization and active fermentative pathways that provide energy for extensive coleoptile elongation under submergence. This trait, referred to as anaerobic germination (AG), is of particular importance for direct-seeded systems, which is becoming increasingly attractive for farmers in both rainfed and irrigated ecosystems due to escalating labor costs for transplanting.
A major QTL for AG derived from the Myanmar landrace Khao Hlan On (KHO), *qAG9-2 (AG1)*, was previously identified and fine-mapped to a ~58 kb region on chromosome 9. The fine-mapped region comprises five putative candidate genes, *AG1-A* to *AG1-E*. Expression studies and sequencing analysis pointed to *AG1-D* as the responsible gene underlying *AG1*. Here we present data supporting this hypothesis. Evaluation of *AG1-D* over-expression in the AG-susceptible mega variety IR64 (*AG1-D*-OX) suggests that *AG1-D* is sufficient to confer AG-tolerance. Coleoptile elongation and amylase activity of *AG1-D*-OX lines are similar to those shown by *AG1* near isogenic lines and significantly higher than those of IR64. Analysis of *AG1-D* promoter-GUS fusion constructs in the KHO background furthermore reveals that *AG1-D* is mainly active in germinating seeds. Particularly tissues associated with the production and secretion of amylases and young sink tissues show a pronounced GUS signal. Taken together our findings imply functions of *AG1-D* in the regulation of starch breakdown.

Keyword : abiotic stress tolerance anaerobic germination starch mobilization gene validation

PE-28

Sub1 locus affects differential transcriptome that regulates the energy consumption and recycling under flash-flooding conditions

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Sub1 locus consisted of three linked *Ethylene Responsive Factors* (ERFs) as the key regulator of flash flooding survival. To elucidate how Sub1 affect tolerance the whole rice performance under stresses, whole transcriptome of the Sub 1 isogenic lines were analyzed under 0, 3 and 7 days of flash flooding compared with KDML105, intolerance line. Principal component analysis (PCA) revealed significant profiles of gene expression under one week flash flooding. A set of 1,100 transcripts representing 1.92% of the whole transcriptome were differentially expressed between the isogenic lines, flooding conditions and their interaction. Using gene ontology (GO) analysis, 1,100 differential transcripts were categorized into starch/sucrose metabolism (12 genes), glycolysis metabolism (15 genes), cell wall expansion genes as same as gene in gibberellins and ethylene synthesis pathways. The most relevant metabolic pathways associated with a list of significant DE (differentially expressed) probes are concerned in starch and sucrose metabolism including glycolytic pathway. Almost genes related to carbohydrate consumption showed low level of gene expression in ISL_Sub1 whereas gene encoding chloroplast targeted beta-amylase in starch degradation expressed highly for 9 times in KDML105. In contrast to gene controlled in conservation of starch in ISL_Sub1, genes encoding glucose-1-phosphate adenylyltransferase and starch branching enzyme (*SBE2.2b)* expressed highly for 3.6x, 9x and 6x, 7x, respectively. Consequently the survival process as carbohydrate consumption and recycling in ISL_Sub1 may be an important key for tolerance mechanism regulated by Sub1genes under flash-flooding stress and can be able to continue growing after several days in flood conditions.

Keyword: Sub1 region, flash flooding, expression profiling

PE-29

Comparative transcriptomics analysis of TNG67 and TCN1 rice seedlings with contrast chilling stress responsiveness

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Low temperature is one of the most important factors that limit the quality and quantity of rice production. Chilling tolerance at the seedling stage is an important agronomical trait for stable rice yield. Thus, there is an increase demand on dissecting the molecular mechanism of chilling tolerance in rice seedling. To investigate chilling tolerance mechanisms of rice, here we reported a comprehensive transcriptome analysis of two rice genotypes (chilling-tolerant TNG67 and chilling-sensitive TCN1) with contrast chilling responses. Time-series of global gene expression analysis under chilling stress revealed that either in shoot or root, there were more differentially expressed genes (DEGs) in TNG67 than in TCN1. However; the number of DEGs increased in TCN1 after subsequent recovery. The tolerant cultivar (TNG67) has an intrinsic mechanism to cope with chilling stress and quickly regain the homeostasis when recovered from stress. In addition, microarray analysis showed that the genes involved in the perception, biosynthesis, degradation and response of plant hormones were differential expressed between these two cultivars, and most obviously related to JA- biosynthesis and response. Further analysis of either up- or down-regulated genes showed that more transcription factors (TFs) genes were identified in TNG67 under chilling treatment as compared with TCN1. These TF genes including *WRKY*, *NAC*, *MYB*, *Zinc-finger* genes expressed specifically at the early stage of chilling stress in TNG 67. The shoot and root-specifically expressed TF genes in TNG67 further indicated that tissue-specific regulons which mediate different bio-pathway may play a role in rice chilling resistance. Taken together, this study identified important hormone-regulated and TF genes that may participate in the chilling tolerance of TNG67.

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Keyword : rice seedling, cold, microarray

PE-30

Toward the understanding of genetic architecture for cold tolerance among the local population in the northern-limit of rice cultivation

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Cold temperature at the reproductive stage (called the booting stage) causes the failure of microspore development, leading to yield reduction. Rice productions in Hokkaido, the northernmost region of Japan and one of the northern-limits of rice cultivation in the world, have serious damage by cold temperature. Due to much effort of rice breeding programs, current cultivars exhibit high level of cold tolerance. Many QTL studies concerning on exotic germplasm with cold tolerance have been reported. However, the achievement of the breeding programs in Hokkaido is independent with such exotic germplasm. This achievement is the results of continuous selection using only the local population. Exotic germplasm to widen the genetic diversity carry many undesirable traits for the local cultivars. Plant breeding is preferred to the local population with narrow genetic diversity rather than exotic germplasm with wide genetic diversity? What contribute to this achievement? To answer this question, we are trying to understand the genetic architecture of cold tolerance among the local population in Hokkaido. A panel of 64 cultivars was selected based on the pedigree in the breeding programs in Hokkaido, then cold tolerance was evaluated using the 19℃-water irrigation system. Furthermore, the developments of pollen and anther were observed using the microscope. This panel is useful for the understanding of the genetic architecture of cold tolerance.

Keyword : cold tolerance, local cultivar,

PE-31

Analysis of pollen-specific gene expression and phenotypic observation in several rice varieties subjected to heat stress

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The recent trends of global warming and climate change has become more difficult to predict and contain, rendering the productivity in agriculture worldwide to receive much vulnerable to various abiotic stress conditions including drought, cold, and heat. As a preliminary step towards developing a heat-tolerant *japonica* rice through molecular breeding, we examined expression of selected pollen-specific genes after subjecting several rice varieties to heat stress. In heat-sensitive *japonica* varieties of rice that we tested, all these genes were down regulated upon heat treatment. However, the expression of some, but not all, of these pollen-specific genes were much less sensitive to the heat stress treatment in heat-tolerant *indica* varieties, suggesting possible functional implication of these genes in conferring heat tolerant phenotype during anthesis of these varieties. Aberrations observed in panicle development as well as in pollen viability incurred by the heat stress treatment were also monitored and the result showed a close correlation overall with the reduction of these transcripts under heat stress. Therefore, these genes, together with the ones involved in the regulatory network for the expression of them, could serve as candidates for useful markers with which molecular breeding of heat tolerant japonica rice can be facilitated.

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Keyword : pollen development heat stress rice panicle heat-specific transcription RT-PCR

PE-32

Association analysis of seed longevity in rice (*Oryza sativa* L.) under conventional and high-temperature germination conditions

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Seed longevity varies considerably in cultivated rice, but the underlying mechanism of longevity is not well understood. To measure seed longevity, we performed an aging treatment at 45ºC on seeds maintained at 14% moisture content for 14 days. We measured the percentage germination of both treated and normal seeds at 25ºC as a control of seed longevity using four replications over 2 years. In total, 140 accessions from a core collection with diverse origins were genotyped using 204 SSR markers, which distributed into 12 chromosomes, to identify marker–trait associations with seed longevity. An analysis of the population structure revealed four subgroups. The r^2 values ranged from 0.0 to 0.8901 for all intrachromosomal loci pairs, with an average of 0.0773. Linkage disequilibrium (LD) between linked markers decreased with distance and displayed a substantial drop in LD decay values between 20 and 50 cM. Marker–trait associations were investigated using a mixed linear model approach, considering both population structure (Q) and kinship (K). Twelve marker–trait associations ($P < 0.01$) were common between the two germination treatments and over the 2-year study, explaining more than 10% of the total variation. These 10 different markers were distributed on five chromosomes. The significant associated SSR markers identified will be useful to seed-bank managers to ensure collections are maintained at high levels of viability to avoid loss of genotypes from the population and for marker-assisted selection.

Keyword : Rice • Seed longevity • Linkage disequilibrium • Population structure • Association mapping

PE-33

Glutamine synthetase (GS) in rice enhances tolerance to cadmium and abiotic stresses

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Glutamine synthetase (GS) (EC 6.3.1.2) is an enzyme that plays an essential role in the metabolism of nitrogen by catalyzing the condensation of glutamate and ammonia to form glutamine. Exposure of plants to cadmium (Cd) has been reported to decrease the GS activity of maize, pea, bean, and rice. To better understand the function of *GS* gene under cadmium stress in rice, we constructed a recombinant vector with *GS* under the control of CaMV 35S promoter and OCS terminator in pART vector and transformed using *Agrobacterium tumefaciens*. The expression patterns and physiological effect both in *GS* overexpressing plants and wild type under cadmium stress and abiotic stress conditions were then investigated. Although a decline in GS enzyme activity and mRNA expression were observed for both plants under cadmium solution, the decrease however, is significantly higher in the wild type compared to the transgenic. Similarly, under low nitrogen treatment, GS-OX lines demonstrated a relatively higher expression level compared to the WT. This was further validated by the higher mRNA expression and GS enzyme activity in most transformed lines. Moreover, glutamine reductase (GR) enzyme showed lower or no malondialdehyde (MDA) activity after 10 days of Cd stress. With these results, it can be inferred that overexpression of *GS* in rice modulated the expression of enzymes responsible for membrane peroxidation that may result to the death in plants.

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Keyword : OsGS, cadmium stress, transgenic, rice

PE-34

Transgenic rice tolerant to low iron availability in a calcareous soil harboring combination of the mutational reconstructed ferric reductase gene and Fe-deficiency inducible transcription factor, OsIRO2

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Iron (Fe) deficiency is a critical agricultural problem especially on calcareous soil, which is widely spread in the world. Rice takes up Fe(II) by OsIRT1 transporter from soil (Strategy I-related system) and also takes up Fe(III) by phytosiderophore-based system (strategy II system). However, rice is susceptible to low Fe condition because of low Fe(III) reduction activity and low phytosiderophore secretion ability. Previously, we produced transgenic rice plants expressing the mutational reconstructed yeast ferric reductase,refre1/372 under the control of *OsIRT1* promoter. This transgenic rice showed higher Fe(III) chelate-reductase activity and Fe deficiency tolerance[1]. We also produced transgenic rice over-expressing Fe-deficiency inducible basic helix-loop-helix transcription factor, *OsIRO2*, which regulates various genes related to Strategy II Fe uptake system including *OsNAS1*, *OsNAAT1*, *OsDMAS1*, *OsYSL15* and *TOM1*. This transgenic rice enhanced phytosiderophore secretion and Fe deficiency tolerance[2]. In the present research, we produced transgenic rice plants which possess both *OsIRT1* promoter-*refre1/372* and *35S* promoter-*OsIRO2* (Refre1/372-OxOsIRO2 lines) to enhance both Strategy I Fe(III) reductase activity and Strategy II-based Fe uptake in rice. Refre1/372-OxOsIRO2 lines exhibited enhanced tolerance to Fe deficiency in calcareous soils compared to non-transgenic line and previous transformants with single introduction of *OsIRT1* promoter-*refre1/372* or *35S* promoter-*OsIRO2*. Refre1/372-OxOsIRO2 lines also showed higher yield performance. Our results show that combination of enhancement of two Fe uptake systems in rice effectively contributes to further tolerance to low Fe availability in calcareous soil.

[1] Ishimaru et al. (2007) Proc. Natl. Acad. Sci. USA 104: 7373-7378.
[2] Ogo et al. (2011) Plant Mol. Biol. 75:593-605.

Keyword : Iron transgenic rice calcareous soil OsIRO2 ferric chelate reductase

PE-35

Characterizing the response of rice to iron excess

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Iron (Fe) is an essential micronutrient for all plants. The mechanism of Fe transport in plants and genes involved in Fe transport under conditions of iron deficiency has been extensively characterized. On the other hand very little is known how plants manage to cope with excess Fe. The expression of rice vacuolar Fe transporter (*OsVIT1*) increases under Fe excess conditions. We characterized *OsVIT1* knockout (*osvit1-1*) and *OsVIT1* over expression (*OsVIT1-1*) line. *osvit1-1* plants accumulated less Fe in shoots when grown under normal conditions, while the accumulation of Fe was comparable to WT plants under Fe-deficient conditions. The accumulation of Zn, Cu and Mn also significantly changed in shoots of *osvit1-1* plants grown under normal growth conditions. The growth of *osvit1-1* plants was also slow compared to WT plants. On the other hand, *OsVIT1-1* plants accumulated more Fe in roots and shoots when grown under Fe sufficient conditions. The accumulation of Fe also significantly changed in *osvit1-1* and *OsVIT1-1* seeds. The expression of *OsVIT1* is higher in knock down plants of rice mitochondrial Fe transporter (*mit-2* plants) and interestingly the accumulation of Fe in *mit-2* seeds was somehow similar to *OsVIT1-1* seeds. These results suggest that the subcellular Fe trafficking is important for plant Fe homeostasis and distribution.

Keyword : iron, vacuolar iron transport, iron homeostasis

PE-36

OsNRAMP5-RNAi rice accumulates higher cadmium and contributes to rapid and efficient cadmium extraction from paddy field

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Cadmium (Cd) is one of toxic metals for humans and causes serious health problems. Cd accumulates in human body through the food chain, and rice is a major source of Cd for Asian people. Therefore, it is important to reduce the Cd content of rice grains. Phytoremediation is a promising method to decontaminate soil Cd using plants. Rice is a good candidate for Cd phytoremediation due to its adaptation to a wide range of environmental conditions, large biomass, and well-established cultivation and harvest methods. In rice, there is genotypic variation in the Cd levels of shoots, and Anjana Dhan is one of the highest Cd accumulating cultivars among world rice collections.

OsNRAMP5 transports Cd in addition to manganese and iron. When *OsNRAMP5*-RNAi Anjana Dhan rice (A5i) was grown in the presence of Cd, Cd content in the shoots was higher than wild type (WT). In the roots of A5i plants, the expression of *OsIRT1* and *OsNRAMP1* was higher compared to WT. Furthermore, we conducted field trials at the experimental paddy field in Korea (0.43 mg Cd kg^{-1} dry weight of soil, as determined with 0.1 N HCl extraction). A5i plants showed normal growth with no significant difference to WT in shoot weight. Cd content in the shoots of A5i was up to 2.0 times higher than that of WT. Rapid and efficient Cd removal from paddy field using A5i plants will contribute to safer food production.

Keyword : cadmium, OsNRAMP5, phytoremediation, transporter

PE-37

Rice plasma membrane intrinsic proteins are involved in transport and providing tolerance to Boron toxicity

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Boron (B) toxicity is responsible for low cereal crop production in a number of regions worldwide. In this report, we characterized two rice genes encoding Plasma Membrane Intrinsic Proteins (PIPs) for their involvement in B permeability and tolerance. Transcript analysis demonstrated that the expression of OsPIP1 and OsPIP2 were downregulated in shoots and strongly upregulated in rice roots by high B treatment. Expression of both OsPIP1 and OsPIP2 in yeast HD9 strain lacking Fps1, ACR3, and Ycf1 resulted in an increased B sensitivity. Furthermore, yeast HD9 strain expressing OsPIP1 and OsPIP2 accumulated significantly higher B as compared to empty vector control, which suggests their involvement in B transport. Overexpression of OsPIP1 and OsPIP2 in Arabidopsis imparted higher tolerance under B toxicity. Arabidopsis lines overexpressing OsPIP1 and OsPIP2 showed significantly higher biomass production and greater root length, however there was no difference in B accumulation in long term uptake assay. Short term uptake assay using tracer B (^{10}B) in shoots and roots demonstrated increased ^{10}B accumulation in Arabidopsis lines expressing OsPIP1 and OsPIP2, compare to wild type control plants. Efflux assay of B in the roots showed that tracer B was effluxed from the Arabidopsis transgenic plants overexpressing OsPIP1 or OsPIP2 in the initial one hour of assay. These data indicate that OsPIP1 and OsPIP2 are involved in mediating B transport in rice and highly useful in developing B tolerant crops for enhanced yield in the areas affected by high B toxicity.

Keyword : plasma membrane intrinsic protein, boron, rice

PE-38

Evaluation of ferritin and iron transporter proteins in biofortified rice

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Biofortification of rice expressing high levels of iron storage and transporter proteins is one of the strategies for addressing nutritional disorder due to micronutrient deficiency in human diet. We have developed transgenic IR64 megavariety expressing ferritin, an iron storage gene, and nicotianamine synthase 2 (OsNAS2) and yellow stripe like (OsYSL), iron transporter genes. . Analysis of the protein sequences and eventual expression of these proteins would provide indications on food safety and also on the resulting phenotype of the transgenic plants. Bioinformatic analysis to compare the protein sequences with sequences of protein allergens permits the evaluation on possible allergic cross-reactivity of the expressed protein in transgenic rice. Iron storage from soybean, ferritin and rice iron transporters, OsNAS2 and OsYSL protein sequences were analyzed using the database in AllergenOnline. Overall search for fused protein sequences of SoyferH1, OsNAS2, and OsYSL proteins showed no significant match with any known allergen. The results suggest that these proteins have no potential cross reactivity to known allergens according to Codex standard. Western blot, and enzyme linked immunosorbent assay (ELISA) have also shown that these proteins were produced to the correct size and had increased level of these proteins in the transgenic rice plants. Analysis also shows that there is good correlation between transcript level, protein level and iron concentration in polished seeds of the transgenic events. Based on the data using high throughput screening by ELISA, lines were selected for phenotypic studies in the succeeding generations.

Keyword : bioinformatics, ferritin, iron transporters

PE-39

Identification of the chromosomal integration site in rice genome using *Zinc finger nucleases*

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For efficient production of transgenic crops the transformation technology plays a very important role. The challenges in plant transformation using *Agrobacterium*-mediated gene transfer and bombardment are multiple transgene copies, random integration and variable expression which can be overcome by integrating the transgene into a predetermined genomic location. Day et. al. (2000) results have shown that a transgene can be delivered into a specific chromosome position; this will allow selection of specific target site for a consistent and higher level of transgene expression. Therefore, the ability to achieve site-specific manipulation of the rice genome can improve the expression of the transgenes as it is highly dependent on the locus of integration. In the present study, we transformed IR64 mega variety by *Agrobacterium*-mediated gene transfer with a modified zinc finger gene construct pRCS2. [KAN][hsp.QQR][QQR-TS∗::GUS] plasmid (Tovkach et. al. 2010) harboring. High expressor lines with a strong GUS marker gene expression were selected and subjected to TAIL PCR. Based on the flanking sequence we are currently analyzing high expressing sites in the rice genome.

Keyword : zinc finger nuclease site specific Agrobacterium-mediated flanking sequence

PE-40

Varietals development for alleviating impacts of global warming during reproductive and seed production stages : Screening for high temperature tolerant rice mutation lines by spikelet fertility.

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Spikelet fertility (seed-set) is an important component of yield that is sensitive to high temperature. Thus, identifying and developing high temperature tolerant cultivars will be an important task for rice breeders to meet the demand for food in the future climates. The objectives of this research were to understand the impact of high temperature stress on rice and screening rice mutant lines for high temperature tolerance. The total of 1,500 M4 lines for Jao Hom Nil mutants (M4) were treated at high temperature (average temperature during the day above 42°C, 6h) at booting stage (R1) to physiological maturity (R9) and natural temperature (average temperature during the day below 35°C,) was used as control. High temperature significantly decreased spikelet fertility to a variable extent. A subset of 236 lines were selected for evaluation of spikelet fertility , 11 M4 lines were tolerance with spikelet fertility in a range of60-69% , while 159 lines were moderately tolerance at range of 40 – 59% spikelet fertility, and 66 lines showed susceptible in a range of 20 - 39% spikelet fertility.The list of control cultivated varieties, Phitsanulok 2, Suphan Buri 1, Riceberry, Jao Hom Nil and Sinlek were on average 26.01%, 30.18%, 21.31%,16.83% and 0% spikelet fertility, respectively. There is a potential for genetic improvement for spikelet fertility and seed set under high temperature stresses.

Key words : high temperature, rice, spikelet fertility.

PF-01

RNA-directed DNA methylation is involved in regulating photoperiod-sensitive male sterility in rice

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Photoperiod-sensitive male sterility (PSMS) is a valuable germplasm for hybrid rice breeding. Recently, we cloned *pms3*, a locus controlling PSMS, which encodes a long non-coding RNA called LDMAR required for normal male fertility of the rice plant under long-day conditions. Increased methylation in the promoter of LDMAR in the PSMS rice (Nongken 58S) relative to the wildtype (Nongken 58) reduced expression of LDMAR leading to male sterility under long-day conditions. In this study, we identified a siRNA named Psi-LDMAR in the LDMAR promoter region that was more abundant in Nongken 58S than in Nongken 58. We showed that Psi-LDMAR was derived from *AK111270*, which was transcribed from the sense strand of the LDMAR promoter, and its 3'-end had a 110-base overlap with the 5'-end of LDMAR. Overexpressing *AK111270* in Nongken 58S greatly enriched Psi-LDMAR, which induced RNA-directed DNA methylation in the LDMAR promoter, and repressed the expression of LDMAR. Reduction of LDMAR in Nongken 58S changed the critical-day length for fertility recovery and delayed the fertility recovery under short-day conditions. This result added to our understanding of the molecular mechanism for PSMS.

Keyword : *Oryza sativa*; siRNA; Psi-LDMAR; epigenetics; hybrid rice.

PF-02

Structural basis of a plant histone H3 Lysine 4 demethylase required for cell division

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Histone lysine methylation is an important epigenetic modification in regulating chromatin structure and gene expression. Histone H3 lysine 4 methylation (H3K4me), which can be in a mono-, di-, or trimethylated state, has been shown to play important roles in gene expression involved in plant development control and stress adaptation. However, the resetting mechanism of this epigenetic modification has yet to be fully understood. In the present work, we identified a JmjC domain-containing protein, JMJ703, as a histone lysine demethylase that specifically reverses all three forms of H3K4me in rice. Loss-of-function mutation of the gene affected cell division and plant growth, which may be related to increased expression of cytokinin oxidase genes in the mutant. Analysis of crystal structure of the catalytic core domain (c-JMJ703) of the protein revealed a general structural similarity with mammalian and yeast JMJD2 proteins that are H3K9 and H3K36 demethylases. Key residues that interact with cofactors Fe(II) and N-oxalylglycine and the methylated H3K4 substrate peptide were identified and were shown to be essential for the demethylase activity *in vivo*. Although the overall structure of c-JMJ703 was similar to that of JMJD2 proteins, several key residues of the a-helix rich region involved in binding to substrate peptides presented distinct structural features, which may be involved in enzymatic specificity for histone H3K4 demethylation.

Keyword : histone demethylase, crystal structure, rice

PF-03

DNA methylation biomarker of 5-methyltryptophan-resistant rice mutants

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DNA methylation is a common and rapidly evolving epigenetic mechanism among higher eukaryotic organisms with complex genomes. Cytosine residues in CG and CNG sequences are the main targets for methylation by DNA methyltransferases. In these studies, we introduce a method for the fast and accurate analysis of DNA methylation based on bisulfate-treated DNA. The target region is PCR amplified using T7 RNA polymerase promoter-tagged primer. A subsequent in vitro transcription leads to a transcript which contained methylated cytosines before bisulfate treatment. In PCR-based methylation assay, PCR amplification of OASA2D CpG Island occurs only when the sites are methylated, and therefore uncut by the two methylation sensitive enzymes. Also the sensitivity of PCR amplification after bisulfate modification was monitored by mixing different proportions of unmethylated DNA and methylated DNA from 5-methyltryptophan resistance rice mutants. Therefore the global DNA methylation pattern of a given region can be very easily determined by enzymatic digestion of PCR products. In addition, more precise mapping of methylation patterns can be performed by cloning and sequencing PCR products.

Keyword : DNA methylation, 5-methyltryptophan, rice mutants

PG-01

Simulation of genome structure and power of QTL detection in rice multi-parent advanced intercross lines

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In rice, many QTLs are localized in specific genomic regions (QTL clusters) that often include naturally occurring allelic variations in different genes. Furthermore, several genes controlling the same trait may be localized in the same QTL cluster, limiting the range of phenotypic variation in a breeding population. Multi-parent advanced intercross lines are powerful tools to resolve this issue. Here, we used computer simulation to investigate the changes in genome structure and the power of detection of linked QTLs in 8-way multi-parent advanced intercross lines. The number of genome segments linearly increased with increases in the number of extra generations of outbreeding. Average and median genome segment length decreased dramatically over the first 5 to 6 outbreeding generations but more slowly with further outbreeding. Without extra outbreeding, small QTL clusters (~15 cM) without any recombination were retained in about half of the individuals. After 5 outbreeding generations, the percentage of genome segments without recombination was <25%. In a population without extra outbreeding, if the QTLs were linked in repulsion phase (additive effects in opposite directions) in the parental genome, the detection power was less than half that for unlinked QTLs. If the interval between two QTLs was >10 cM, more than 5 outbreeding generations dramatically increased the power of detection. In the case of coupling-phase linkage (additive effects in the same direction), extra outbreeding slowly decreased the power of detection. Thus, our simulation study indicated the effectiveness of 5 to 6 outbreeding generations in the construction of multi-parent advanced intercross lines.

Keyword : QTL, Simulation, Advanced intercross lines

PG-02

Heterosis QTLs for grain yield and yield-related traits in *indica-japonica* crosses of rice

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Heterosis describes the increased performance of F_1 hybrid plants in terms of increased biomass, yield, vegetative growth rate, and tolerance of biotic and abiotic stresses as compared with their genetically different inbred parents. Two sets of rice materials, 166 RILs derived from a cross between Milyang 23 (a Korean *tongil-type* rice) and Tong 88-7 (a temperate *japonica* variety), and BC_1F_1hybrids derived from crosses between the RILs and the female parent, Milyang 23, were produced to identify heterosis QTLs for yield and yield-related traits in *indica-japonica* crosses. The QTLs were detected from three different phenotype data sets including RILs, BC_1F_1 hybrids, and mid-parental heterosis data set. A total of 57 QTLs were detected for nine traits. Out of eight QTLs identified for yield heterosis, five overlapped with other heterosis QTLs for yield-related traits. Four heterosis QTLs for yield, *gy1.1*, *py6*, *gy10*, and*py11*, were newly identified in this study. We identified a total of 17 epistatic QTLs for yield heterosis. This work was supported by a grant from the Next-Generation BioGreen 21 Program (Plant Molecular Breeding Center, No. PJ009076), Rural Development Administration, Republic of Korea.

Keyword : yield, heterosis, QTL, rice

PG-03

Uncovering the genomic structure of Japanese high-biomass rice cultivars derived from japonica–indica crosses

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High-biomass rice cultivars for food, feed, and forage production in Japan have been developed from crosses between *japonica* and *indica* cultivars. Analysis of the genome structures of the admixed cultivars might reveal regions associated with important agronomic traits such as yield and disease resistance. To uncover the genome structure of high-biomass cultivars, we established a subset of 1152 single-nucleotide polymorphisms (HB-SNP subset) with an even distribution among the 12 chromosomes. We selected this subset by screening 5760 genome-wide SNPs previously found between 14 high-biomass cultivars and Nipponbare. Analysis of genetic diversity among high-biomass Japanese cultivars, their ancestors, and reference cultivars selected from both world and Japanese rice core collections revealed that the HB-SNP subset had high polymorphism information content for the high-biomass Japanese cultivars. A phylogenic tree and genetic structure constructed by using the HB-SNP subset classified the high-biomass Japanese cultivars into 5 clusters (out of 6 total clusters), with varying proportions of major alleles contributed by the *indica* cultivars. In the cluster containing admixed cultivars of both *indica* and *japonica* cultivars, regions of chromosomes 7, 8, 10, and 12 contained high frequencies of major alleles from *indica* cultivars. Genome-wide association mapping of high-biomass cultivars and their progeny detected a significant SNP for blast susceptibility around the *Pi-ta* region on chromosome 12, with the *indica* allele conferring tolerance to blast. These results imply that this genomic region derived from *indica* rice would contribute to blast tolerance in high-biomass cultivars in Japan.

Keyword : High-biomass, *indica*, *japonica*, SNP

PG-04

QTL analysis for some agronomic characters in two rice populations derived from wide-compatibility line

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The HWC-line of rice showed tall culm length, slender grain shape, wide compatibility with both *indica* and *japonica* cultivars. For QTL analysis, two F_2 populations were derived from the crosses between the HWC-line and each of two Korean variety, Dasanbyeo (Korean *Tongil-type* cultivar) and Hwacheongbyeo (temperate *japonica* cultivar), respectively. A total of 190 F_2progeny were developed in each of two F_2 populations, with which two molecular linkage maps were constructed. Eight agronomic characters were measured for QTL analysis in these F_2 populations and parents, revealing 16 M-QTLs and 1 E-QTL for culm length, spikelets per panicle, spikelet fertility, grain length, grain width, grain shape and 100 grains weight. 7 QTLs were newly found in this study. In the F_2 population from another cross, 15 M-QTLs were detected for culm length, panicle length, spikelet fertility, grain length, grain width, grain shape and 100 grains weight, 6 QTLs of which were newly identified. These QTLs would provide central information on putative functional genes related with agronomic characters and expedite breeding new rice cultivar.

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Keyword : QTL, wide compatibility, *indica*, *japonica*

PG-05

Yield evaluation of two-line hybrid rice

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Thailand is the world's largest exporter of rice famous for high quality rice but average grain yields is low. Hybrid rice varieties have shown 15-30% higher yield than inbred varieties, and have also improves several other desirable traits. The temperature-sensitive genic male sterility (TGMS) system has high possibility to increase food production by hybrids. Previously, we evaluated 7 exotic TGMS rice lines controlled by 3 *tgms* genes obtained from International Rice Research Institute (IRRI) for their potential use for hybrid production. In this study, four TGMS lines were selected to use as female parents for two-line hybrid development by crossing with 25 Thai lines to generate F1 hybrids. Forty-five F1 hybrids were planted in two locations in the central areas, and another 13 F1 hybrids were planted in one location in the north. These hybrids were evaluated along with their parents and 3 standard varieties using randomized complete block design (RCBD) with three replications during rice season in 2010. There were 1,6,34 hybrids in one location in the central area, 11,17, 20 hybrids in the other central area, and 6,7,7 hybrids in the north having higher grain yield more than 20% over the 3 best standard varieties, PTT1, PLS2, RD31, respectively. In 2012, forty-three F1 hybrids were evaluated at the three locations using RCBD with three replications to identify suitable parents for developing high yielding hybrid. The results will be discussed.

Keyword : combining ability, two-line hybrid rice, yield

PG-06

Genetic composition of yield heterosis in an elite rice hybrid

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Heterosis refers to the superior performance of hybrids relative to the parents. Utilization of heterosis has contributed tremendously to the increased productivity in many crops for decades. Although there have been a range of studies on various aspects of heterosis, the key to understanding the biological mechanisms of heterotic performance in crop hybrids is the genetic basis, much of which is still uncharacterized. In this study, we dissected the genetic composition of yield and yield component traits using data of replicated field trials of an "immortalized F2" population derived from an elite rice hybrid. Based on an ultrahigh density SNP bin map constructed with population sequencing, we calculated single-locus and epistatic genetic effects in the whole genome and identified components pertaining to heterosis of the hybrid. The results showed that the relative contributions of the genetic components varied with traits. Overdominance/pseudo-overdominance is the most important contributor to heterosis of yield, number of grains per panicle, and grain weight. Dominance-by-dominance interaction is important for heterosis of tillers per plant, grain weight and also has roles in yield and grain number. Single-locus dominance has relatively small contributions in all the traits. The results suggest that cumulative effects of these components may adequately explain the genetic basis of heterosis in the hybrid.

Keyword : dominance, overdominance, epistasis, bin-map, immortalized F2, recombinant inbred intercross

PG-07

QTL mapping of rice biomass-related traits in recombinant inbred lines from crosses between high-yielding cultivars

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Biomass yield (i.e., yield of stem, leaf, and grain) has been identified as an important target in rice breeding, yet we know little about the genetic basis underlying variation in rice biomass. Here, we performed quantitative trait locus (QTL) analysis for 3 biomass traits—plant weight (PW), grain weight (GW), and stem and leaf weight (SLW)—and 12 of their component traits by using recombinant inbred lines (RILs) from crosses between high-yielding cultivars 'Tachisugata' and 'Hokuriku 193'. The study was designed to explore the genomic potential for further increases in rice biomass yield. Composite interval mapping identified 60 significant QTLs underlying the 15 biomass-related traits. These QTLs were found on 8 of the 12 rice chromosomes, and QTLs for multiple traits were found clustered in certain genomic regions. Significant phenotypic correlations (both positive and negative) might be generated by such QTL clusters. A combination of 4 QTLs accounted for 36.0% of PW variation, 3 for 22.3% of GW variation, and 5 for 31.0% of SLW variation. Multiple regression analysis revealed that 42.8% of PW variation was explained by a combination of QTLs for several biomass traits, and the mean of lines that carried alleles with positive additive effects at these QTLs showed an 11.8% increase in PW over that of 'Hokuriku 193', which had a higher mean than 'Tachisugata'. Our results thus suggest that further biomass improvement can be achieved by combining favorable alleles at multiple QTLs.

Keyword : Biomass yield QTL

PG-08

A yeast two hybrid screening to search for protein that interact with RF2, a restorer of fertility of LD-type cytoplasmic male sterility in rice.

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Cytoplasmic male sterility (CMS), which is caused by an abnormal mitochondrial gene, is inability to produce functional pollen. CMS lines are unable to produce seeds by self-pollination, and therefore they are used widely all over the world for effective hybrid breeding. Pollen function is often known to be recovered by *Restorer of fertility* (*Rf*) gene encoded by the nuclear genome. Many *Rf* genes are reported to encode pentatricopeptide repeat (PPR) protein which is involved in processing of mitochondrial RNA. Different from PPR-type *Rf* gene, *Rf2* which is a fertility restorer gene of Lead Rice (LD) type CMS, encodes glycine-rich protein. *Rf2* is, therefore, considered to restore fertility by a novel mechanism. RF2 is expected to function by interacting with other proteins, because RF2 have no motifs except glycine-rich domain (Itabashi et al., 2011). To elucidate the protein that interacts with RF2, we performed yeast two-hybrid (Y2H) screening, using the library made from anther RNA of rice. As a result, we identified four genes (RF2 interacting factors; R2IF1~4). Next, we performed pull-down assay to test *in vitro* interaction. Using *E. coli*-produced recombinant RF2 and R2IF2 (Ubiquitin domain containing protein), we confirmed that RF2 interacted with R2IF2. These results suggest that RF2 functions with R2IF2, and participates in fertility restoration. Pull-down assay for the other three genes is now in progress. This study is partially supported by a Grant-in-Aid (No.2338002) from the Ministry of Education, Science, Sports and Culture, Japan.

Keyword : mitochondria, nuclear, cytoplasmic male sterility, yeast two-hybrid

PG-09

Fine mapping of a quantitative trait loci controlling the number of spikelets per panicle in rice

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Spikelets per panicle (SPP) is one of the most important traits associated with rice yield. In this study, IL28, a near isogenic line (NIL) developed by introgressing chromosomal segments from 'Moroberekan' into 'Ilpumbyeo' showed significantly higher number of spikelets per panicle than the recurrent parent, 'Ilpumbyeo'. Quantitative trait locus (QTL) analysis in 243 F_2 plants derived from a cross between IL28 and Ilpumbyeo indicated that a QTL for spikelets per panicle, *qSPP6* was located in the interval RM3430 -RM20580. The Moroberekan allele increased SPP. The fact that QTLs for panicle length and the number of secondary branches were mapped in the same interval as *qSPP6* appears to indicate that this locus was associated with panicle structure. To map the QTL more precisely, substitution mapping of *qSPP6* using F_3 lines was conducted. Substitution mapping with 41 F_3 lines further narrowed the interval containing not only *qSPP6* for spikelets per panicle but also *qNDW6* for node width to about 680-kb between markers RM20521 and RM20572 based on Nipponbare genome sequence. The locus, *qSPP6* is of particular interest because of its independence from undesirable height and flowering time. SSR markers tightly linked to the *qSPP6* will facilitate cloning of the gene underlying this QTL as well as marker assisted selection for variation in SPP in the breeding program.

Keyword : Spikelets per panicle, rice, quantitative trait locus, pleiotropy

PG-10

Fine-mapping of a QTL for increased total spikelet number, qTSN4, in a tropical *japonica* rice variety

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The development of high-yielding varieties is an important breeding objective in rice (*Oryza sativa* L.). To improve grain yield of an *indica* variety, IR64, introgression lines (ILs; BC_3-derived lines) have been developed using a new plant type (NPT) rice with heavy panicles and low tillering ability from *tropical japonica* type rice varieties. One of the ILs with high total spikelet number per panicle (TSN), YTH326, was selected for genetic analysis. In our previous analysis, QTL for high TSN (*qTSN4*) was detected on the long arm of chromosome 4 using an F_2segregating population derived from a cross between IR64 and YTH326. In this study, a high-resolution linkage map was constructed using 7,996 F_3 plants to identify the detailed location of qTSN4. The candidate region of *qTSN4* was specified between ind8M17-4 and ind8M17-12, representing 18.0-kbp based on the Nipponbare genome sequence. Additionally, leaf width in the segregating population was co-segregated with TSN and the gene for leaf width was located on the same region of *qTSN4*. Furthermore, a near-isogenic line (NIL) for *qTSN4* with the genetic background of IR64 was developed from the IR64/YTH326 population and designated as IR64-NIL5. Compared with IR64, this NIL showed higher TSN, wider leaves, larger roots, and thicker panicle neck. Consequently, this NIL showed higher grain yield in field trial for several seasons.

Keyword : fine-mapping, spikelet number per panicle, grain yield, tropical *japonica*

PG-11

Evolutionary difference of the genes regulating grain size and their function combination in rice

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Grain size is an important agronomic trait for yield determination in rice. Many genes regulating grain size have been mapped or cloned since 21 century. To explore the evolutionary feature and function combination of grain size related genes in rice, deep sequencing of *GW2*, *GS5* and *qSW5* was made in both natural population of 127 accessions (*O. sativa*) and 15 common wild rice accessions (*O. rufipogon*). *GW2* showed a significant positive selection in its genome while *GS5* showed a highly similarity sequence with its progenitor *O. rufipogon*, and *qSW5* genome contained the highest level of unclear diversity. Correspondingly, 10, 10 and 15 haplotypes were constructed in natural population respectively of the three genes. Moreover, the effect of every haplotype was estimated respectively in *indica* and *japonica* subpopulation. Together with *GS3*, the function difference of the 4 gene combinations had been evaluated. *qSW5* and *GS3* were the most prevalent genes in determining grain size, while *GW2* and *GS5* showed modest effect in natural population. These findings provide an insight into the evolutionary feature of the genes regulating grain size in rice and made a flexible utilization of these genes in molecular breeding.

Keyword : rice grain size, evolution, domestication

PG-12

Breeding multi stress tolerance aromatic glutinous rice variety for rainfed lowland rice production in Mekong region coping with climate change

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Rice is an important staple food crop in the Mekong region in Southeast Asia. Climate change has major effect on rice production and threatened food security for people in this region. The consequence of the climate change includes drought, flooding and increased incidence of disease and insect infestation. Rice breeding for multi stress tolerance and wide adaptability is an effective approach that can cope with the adverse effects of climate change. We aimed to develop aromatic glutinous rice variety resistance to blast and tolerance to submergence using marker assisted selection (MAS). F_1 seeds were produced from crosses between jasmine IR57514-introgression lines tolerance to submergence and RD6-blast, a jasmine glutinous rice variety resistance to blast disease. Four selfing were carried out and MAS was performed in each generation to select favorable alleles of five target traits including *wx* for glutinous, *badh2* for grain fragrance, *SSIIa-TT* for low gelatinization temperature (GT), *qBl1* and *qBl11* for blast resistance and *Sub1* for submergence tolerance using *Wx*-Glu-23, Aromarker, SNP2340-41, RM212 and RM319; RM144 and RM1233, and R10783 as foreground selection, respectively. Preliminary submergence tolerance was evaluated in F_3population and result confirmed that this desirable character from IR57514 introgression lines has introgressed to these selected F_3 progenies. Finally, the MAS has identified 22 F_4 homozygous lines carrying favorable alleles ofglutinous ($wx^{RD/RD}$), grain fragrance ($badh2^{IR/IR}$), low GT ($SSIIa\text{-}TT^{IR/IR}$), blast resistance ($qBl1^{RD/RD}$, $qBl11^{RD/RD}$) and submergence tolerance ($Sub1^{IR/IR}$). These elite introgression lines is currently on going for validation of agronomic and yield performance, blast resistance, submergence tolerance and grain quality.

Keyword : Rice, Climate change, marker assisted selection, grain fragrance, blast resistance

PG-13

Molecular Breeding of Rice Variety Sin-Thwe-Latt for Submergence and Salinity-prone Areas in Myanmar

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Salinity and submergence problems commonly occurred not only costal and delta regions but also some irrigated rice growing areas of middle Myanmar and greatly reduce grain yield. Sin Thwe Latt (IR53936-60-3-2-3-1), widely grown across the country due to its high yield and market preference, had been improved by marker assistant selection (MAS) since 2003 to incorporate salt tolerant gene (on chromosome 1) from Pokkali. IR57514 was developed by IRRI and had been identified to be wide-adapted to rainfed lowlands of Mekong region. Later, it was identified as submergence-tolerant variety that carries a *Sub1*region (on chromosome 9). The present study was conducted to acquire Myanmar popular rice variety Sin-Thwe-Latt (IR53936-60-3-2-1) to have with submergence and salt tolerant genes through molecular marker assisted backcrossing strategy. In this regard, BC_3F_4 Yn3220-108-2-3-1, introgression line of IR 53936 (Sin Thwe Latt), was crossed with RGDU 07343-9-13-26, an improved line if IR57514 which carries submergence tolerance, BB resistance and aroma as well. In order to achieve the target traits, 9 lines from BC_2F_2 progenies were selected by using molecular markers, PB7 and 8, R10738, BADH, RM1287 and RM3412 and continued to follow the conducted breeding program. The present study is an on-going research where in MAS and validation for target traits will be done in BC_2F_3 population. Consequently, the existing popular cultivar Sin-Thwe-Latt will become a new cultivar with submergence and salinity tolerant genes without changing its desirable agronomic characters suited to most rice growing areas of Myanmar and quality trait accepted by consumers and market. The results obtained from this program will be beneficial for increasing rice productivity especially salinity and submergence prone areas of Myanmar which may improve farmers' income.

Keyword : Rice, salt and submergence tolerant, Marker assisted backcrossing

PG-14

Marker assisted introgression of three major genes determining cooking quality from Thai jasmine rice into high yielding rice variety, IR57514

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IR57514 is a high yielding, drought tolerant and submergence tolerance rice variety widely adapted to Mekong region. Its cooking quality, however, is unsatisfactory due to non fragrance and hard texture when cooked. Aromatic rice with soft texture is preferable by people in this region. Therefore, we aimed to converse poor cooking quality of IR57514 into jasmine-like cooking quality using marker-assisted backcross breeding. KD571-77, a submergence tolerance jasmine rice introgression line, was used as a donor for good cooking quality traits. Three backcrosses and three selfing were carried out to transfer favorable alleles of *badh2* for grain fragrance, Wx^b for low amylose content and *SSIIa-TT* for low gelatinization temperature from KD571-77 into IR57514 using three functional markers, Aromarker, Waxy and SNP2340-41 as foreground selection, respectively. Twelve $BC_3F_{3:5}$ introgression lines conferring homozygous favorable alleles of *badh2*, Wx^b and *SSIIa-TT* were selected and further evaluated for yield performance and submergence tolerance. The results showed that average grain yield and submergence tolerance performance of these elite introgression lines were not significant difference from the high yielding recipient parent, IR57514. Finally, the $BC_3F_{3:6}$ seeds were validated for grain fragrance and cooking quality. The evaluation results have shown that all elite introgression lines were fragrance, puffy and soft texture when cooked (low AC and GT) similar to the donor jasmine parent, KD571-77. The present study has demonstrated the success of MAB to improve grain quality of IR57514 rice cultivar. The jasmine IR57514 will greatly benefit farmers in the rainfed lowland rice production in Mekong region in Southeast Asia.

Keyword : Rice, cooking quality, submergence tolerance, marker- assisted backcross breeding

PG-15

Pyramiding multiple quantitative trait loci (QTL) for brown planthopper resistance in a high yield variety chainat 1 by marker-assisted selection

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Brown planthopper (BPH) (*Nilaparvata lugens* Stal) is one of the major insect pests of rice (*Oryza sativa* L.) in south and south-east Asia. It causes in yield loss in many areas of Thailand especially the central plain. Chainat1 (CNT1) is widely grown in this irrigated area. The variety was released with BPH resistance, it became susceptible lately. Rice Gene Discovery Unit, Kasetsart University, Kamphangsaen Campus has developed CNT1 with BPH resistance genes from two resistant cultivars. CNT1 (*Bph3*) obtained the resistance gene *Bph3* from Rathu Heenati which was mapped on the short arm of chromosome 6. CNT1 (*Qbph6*, *12*) obtained two quantitative trait loci (QTL) from Abhaya. It is believed that pyramiding genes from different cultivars can increase durability of the resistance to BPH. Parental lines from those crosses were used for crossing through MAS, phenotypic, agronomic and plant type selection. One PCR-base marker was used to select F_1 progenies and three PCR-base markers were used to select resistance alleles for 90 lines of homozygous recombinant F_2. Seedbox screening to evaluate the BPH resistance level of the pyramided lines will be used to select F_3 progenies. Seven F_1 progenies were found to be heterozygous among twelve F_1 progenies derived from those crosses and were self pollinated to generate F_2 plants. Ninety F_2 progenies were derived from those selfing and were used to select with MAS. Our expected result, introgression lines are durability of resistance, good quality and utility in breeding program.

Keyword : BPH, pyramiding, MAS, durability

PG-16

Influence of genetics and environment on the primary metabolite content of cooked rice

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Genetic diversity is a source of variation for health-related secondary metabolites in cooked grain. While breeding for distinct secondary metabolite profiles is a novel goal, neither the genetic regulation nor the heritability of these profiles in cooked food is well understood. Thus, characterizing the regulation of precursor (primary) metabolites is an important step. Here, a metabolomics approach was conducted to assess the influence of genetics, environment, and genotype-environment interactions (GEI) on primary metabolites in cooked rice. The varieties IR64 (an advanced line) and Moroberekan (a landrace), were grown in field and greenhouse environments. Cooked rice metabolites were extracted in an aqueous methanol solvent and detected by gas chromatography coupled to mass spectrometry. Mass spectral database matches identified candidate compounds that differed between the two lines and environments and included amino acids, fatty acids, mono- and disaccharides, inorganic and organic acids, and others. Most metabolites exhibited genotypic, environmental, and/or GEI effects as significant sources of primary metabolite variation. A gene-metabolite network analysis was conducted to characterize the covariance between amino acids and genes with similar enzymatic function, and novel epistatic relationships among genes and primary metabolites are proposed. These data support that diversity in metabolite synthesis genes, genetic background, environmental growing conditions, and interactions among these effects collectively contribute metabolite variation in the cooked grain. Variation in primary metabolism may be implicated in secondary metabolite profiles of the cooked grain, and warrant further investigation to identify factors that affect the heritability of these traits.

Keyword : metabolite profile, cooked rice

PG-17

A rice genomes SNP db for genotyping *Oryza* species and cultivars that are of interest to your country

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Some groups consider genomics is simply the accumulation of sequence data. We believe genomics is using genome-wide DNA identity markers, along with profiles of RNA species expression, as a practical tool to qualify phenotypes related to environment. For rice, sequence data from many projects has opened catalogues of potential and some validated SNPs that show a Genotype Associated to Phenotype [GAP] relationship. Validated GAPs establish genomic selection as a practical component to increase genetic gain. To have real impact in rice breeding, GAPs should validate across many varieties and in many grow-out environments. For phenotypes controlled by complex biological pathways, we believe the analysis power of genome-wide studies on population samples will show which SNP matrix best describe each GAP. The bovine industries are using a GAP approach to great success. The poultry industry is on the way and now wheat, strawberry, soybean and other crops are beginning. These all have foundation in a strong SNP db, tested genotypes and phenotype data for reference populations. Linking genotyping technology to a Rice Genomes SNP db containing millions of markers will provide breeders and researchers the information to allow genome-wide scans, trait fine mapping and discovery of GAPs in *Oryza* species and cultivars that are of interest. We built the Axiom® Bovine SNP db as an open-source digital library. We built the Axiom® Chicken SNP db. Our poster will demonstrate how you can build an Axiom® Rice SNP db for access to functional genotyping. Do you want to participate?

Keyword : SNP-database, Genotyping, Breeding, GWAS, Axiom.

PG-18

High-throughput SNP genotyping for breeding applications

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Recent advances in single nucleotide polymorphism (SNP) marker technologies can enable rapid and cost-effective genotyping of large numbers of DNA samples for breeding applications. We are currently developing a high-throughput SNP genotyping workflow at IRRI to offer as a service to support the needs of rice breeding programs within the Global Rice Science Partnership. An improved sample preparation workflow is being optimized to increase the efficiency of rice leaf tissue sampling, DNA extraction, and DNA quality control in preparation for SNP genotyping. Validated markers from the 44K SNP chip developed at Cornell University are being used to select subsets of informative SNPs for different germplasm groups and genome regions. A 384-plex SNP set for *Indica* germplasm and a 384-plex SNP set for *Indica/Japonica* material are being routinely used for genetic diversity analysis, QTL mapping, and marker-assisted backcrossing. However, more work is needed to develop SNP fingerprinting databases to support variety identification and as a rapid quality control test to detect errors in seed identities. Sets of 24 and 96-SNP Fluidigm chips are also being tested to enable rapid, low-cost assays for running markers targeted to specific chromosomal regions and genes controlling traits of interest. Functional SNPs and allele-specific SNP haplotypes are being validated for genes controlling important traits to provide custom sets of marker packages tailored to different rice breeding programs. Genotyping by sequencing (GBS) using different sequencing platforms is also being tested to provide high-resolution genome scans for association mapping, tracking introgressions and for genomic selection.

Keyword : SNP genotyping, functional markers, breeding

PG-19

MAGIC: A new genetic resource for mapping and breeding

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Dissecting the genetic variants of natural variation that underlie in key important traits in rice remains unresolved and has been very challenging. To harness natural allelic variation comprehensively in breeding while allowing the genetic dissection of complex traits individually, we generated a new genetic resource called multi-parent advanced generation intercross (MAGIC) population using, as the founder parents, eight elite indica and eight japonica cultivars with desirable traits such as high yield, good grain quality, and tolerance to a suite of stresses. We have developed 4 multi-parent populations: *indica* MAGIC (8 *indica* parents); MAGIC plus (8 *indica* parents with two additional rounds of 8-way F1 intercrossing); *japonica* MAGIC (8 *japonica* parents); and the Global MAGIC (16 parents – 8 *indica* and 8 *japonica*). Genome-wide association mapping using genotyping-by-sequencing of early generation population was able to tag known genes/QTLs such the *Sub1* for submergence tolerance, *qBR9.1* for blast disease resistance, and *Saltol* for salinity tolerance, with SNP markers within or very close to the gene/QTL itself. Moreover, several novel QTLs for these same traits were also identified. Agronomic and yield data from $S_{2:4}$ indica MAGIC lines tested in multi-environments suggest the transmission of favorable alleles from the donor parents and a good combination of agronomic traits for breeding. The new and diverse genetic resource promises to be a valuable source of breeding-ready materials for the extraction of new commercial varieties with multiple economically important traits as well as a rich resource for fine mapping QTLs and/or combining QTLs for multiple traits in rice.

Keyword : Multi-parent Advanced Generation Intercross, Genotyping-by-sequencing, Genome-wide association mapping, Genetic resource, Allelic variation

PG-20

Detection of genetic polymorphism among some rice varieties by URP-PCR

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Rice(*Oryza sativa* L.) is the model monocot plant to study cereal genomics. Most importantly, it is the main staple food of choice for almost all of our Myanmar people. The present study was designed to detect some genetic polymorphisms among some rice varieties at genome-wide level by arbitrary priming with Universal Rice Primers (1F, 2F and 4R. The genetic similarity among selected rice accessions ranges from 30.8% to 77.3%. The observed phenetic tree shows 14 rice samples in cluster A and 5 samples in cluster B showing the potential of URP primers in the study of genetic diversity and relationship among rice varieties in resource-limited laboratories

Keyword : rice, genetic polymorphism, universal rice primers

PG-21

Control of gibberellin levels by point-mutated Gibberellin 2-Oxidases enhances yield and stress tolerance in rice

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Bioactive GAs are inactivated by C_{19} and C_{20} gibberellin 2-oxidases (GA2oxs). Class C_{20} GA2oxs contain three unique conserved motifs as compared to class C_{19} GA2oxs. Previous studies indicate that ectopic overexpression of C_{20} GA2oxs results in less severe GA deficient phenotypes than expression of C_{19} GA2oxs in rice. In the present study, to investigate the function of three conserved motifs in C_{20} GA2oxs and to more precisely control GA levels for optimal plant growth and productivity, point mutations in ten amino acids within three conserved motifs was created and analyzed in a representative C_{20} GA2ox, GA2ox6. Five effective mutations were found to reduce GA2ox6 enzymatic activity to different extents, which led to various degrees of GA deficient yet beneficial agronomic traits, including semi-dwarfism and increased tiller number, higher chlorophyll density, reduced shoot to root ratio, and enhanced water use efficiency in transgenic rice. GA deficient transgenic rice also exhibited altered leaf architectures that are associated with abiotic stress tolerance. Among five GA2ox6 mutants, mutant E conferred moderate while mutant K conferred significant enhancement of stress tolerance to dehydration, salt, heat and cold stresses in transgenic rice. In field conditions, mutant E led to 32% increase while mutant K slight reduction in grain yield in transgenic rice. Our studies demonstrate that increase in grain yield and stress tolerance could be achieved by controlling GA levels through ectopic overexpression of defective GA2ox in rice.

Keyword : gibberellin 2-oxidases, abiotic stress tolerance, grain yield

PG-22

Molecular marker application to incorporate salinity and blast tolerance in West Africa rice varieties

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Rice production in Africa is affected by many biotic (blast, BLB, RYMV) and abiotic stresses (salinity, drought, heat and cold) which threaten the sustainability of rice production among farmers. Tolerance to most of this stresses are controlled by a complex of characters resulting from the interaction of many quantitative component traits. Marker -based genetic studies has permitted the identification of reliable QTLs for many abiotic and biotic stresses. To demonstrate the value of marker-aided selection to manipulate QTLs, we described some examples of introgression of genes/QTLs for blast and salinity tolerance into elite Africa germplasms (Sahel 108, RASSI, Kogoni-90-1 and NERICA-L-19).The first study targeted blast resistance from 2 genes (Pb1 and Pi21) which have been reported to have broad spectrum of resistance under field conditions to Africa strains from 2 japonica varieities; MODAN (Pb1) and Owarihatamochi (Pi 21) were introgressed into some Africa varieities through backcrossing with selection based on marker information alone. The evaluation of BC2F2 families is yet to be conducted under field condition. For the second study, targeting early seedling stage salinity tolerance QTL (Saltol), we used 3 cycles of backcross with selection based on marker information and field evaluation. The BC3F2s are currently been characterized for other agronomic performance. These approach shows that much progress can be made by combining both markers selection and evaluation under the stress condition after each cycle of hybridization. The long term goals are to develop a higher level of tolerance to salinity and blast in high yielding African rice varieties

Keyword : rice, salinity tolerance, blast tolerance, MAS, QTL, genes

PG-23

Genetic diversity of aromatic rice landraces in Thailand and Myanmar as revealed by isozyme grouping and F-AFLP

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Isozyme grouping by a set of 19 SSR markers distributed on the 12 chromosomes together with DNA fingerprinting can be used to study biodiversity and evolution of local aromatic varieties. 480 varieties from The National Seed Storage Laboratory Genetic Resources (Genebank), Pathum Tani Rice Research Center and 100 Myanmar varieties include isozyme group I and V were used to study diversity by F-AFLP and isozyme grouping by SSR markers. For pre-genotyping, multiplex gene specific markers on BADH2 include 5'SSR, 3'Indel and three functional markers, 8bDel, 3bIns, 7bDel, were employed. There were 315 out of 480 varieties contained 8 bp deletion allele in Thai rice and 20 out of 100 varieties in Myanmar rice. From clustering result, 250 varieties were selected to perform isozyme grouping and F-AFLP studies. Result showed that, most of Thai local varieties belong to isozyme group I and can classified into 5 groups by F-AFLP. Aromatic rice also included in all AFLP groups. From the study, we can group local aromatic varieties at the whole genome level and compared with isozyme grouping by SSR markers for understanding diversity of aromatic landraces rice in Thailand.

Keyword : Aromatic rice, Biodiversity, Isozyme group

PG-24

Genetic structure of Thai rice and rice accessions obtained from the International Rice Research Institute

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Although the genetic structure of rice germplasm has been characterized worldwide, few studies investigated germplasm from Thailand, the world's largest exporter of rice. Thailand and the International Rice Research Institute (IRRI) have diverse collections of rice germplasm, which could be used to develop breeding lines with desirable traits. To better understand the genetic structure and relationships among these germplasm, we used 19 InDel markers selected from 98 markers to evaluate 43 Thai and 57 IRRI germplasm, including improved cultivars, breeding lines, landraces, and 5 other *Oryza* species. The Thai accessions were selected from all rice ecologies. The IRRI accessions were groups of germplasm having agronomic desirable traits. Most of the tested InDel markers were genes with diverse functions. The results showed that Thai and IRRI germplasm were significantly different. Thus, they can be used to broaden the genetic base and trait improvements. Cluster, structure, and differentiation analyses showed concordant results having six distinct groups, in agreement with their development, and ecologies.

Keyword : Genetic diversity, Germplasm evaluation, InDel marker, *Oryza sativa*, Population structure, Temperature sensitive genetic male-sterile

PG-25

Effect of gamma irradiation on sorghum *Sorghum bicolor* (L.) Moench genome

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Sorghum [*Sorghum bicolor* (L.) Moench] is a model C_4 crop with NADP-ME type of photosynthesis and well defined Kranz leaf anatomy. It is known for high productivity and tolerance to various stresses such as drought and heat. It has an intermediate genome size of 730 Mb. Its whole genome has been sequenced; however, the genome has not been well annotated. To create variations in complex traits and hence to identify genetic factors, mutation is a widely used method. The effect of various doses of gamma mutation on the sorghum whole genome is not known. In this study, mature seeds of sorghum, BTx623 accession were treated with different doses of gamma rays [600 Gy, 500 Gy, 400 Gy, 300 Gy, 200 Gy and Zero Gy (wild type, WT)]. Radiation of 200 GY on mature seeds was too weak to cause major phenotypic alterations and 600 Gy decreased germination by 50 %, only 40% of the germinated seedlings survived and 20 % of the survived seedlings were fertile with low number of seed set per panicle. One representative M2 plant from each of 500 Gy, 400 Gy, and 300 Gy mutant populations and one WT were sequenced using next generation sequencing technology. The four genome sequences were aligned against the reference genome sequence of BTx 623. The number of insertions, inversions, deletions, inter-chromosomal translocations and single nucleotide polymorphism were analyzed. Effects of mutation at gene levels are being analyzed.

Keyword : Indels, Kranz Anatomy, Mutation, Next Generation Sequencing, Single Nucleotide Polymorphism

PG – 26

Molecular breeding of high miraculin contents in rice (*Oryza sativa L.*)

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We constructed a cultivation system with a controlled light period, light intensity, temperature, and CO(2) concentration for mass production of the taste-modifying protein miraculin from transgenic rice. The rice plants exhibited normal growth and produced over 170 g of fresh weight (FW) fresh weight of seedlings, with the recombinant miraculin concentration reaching up to 60μg/ FW of rice seedlings. The recombinant miraculin content of transgenic rices was compared to that of plants grown in a pot of greenhouse. The recombinant miraculin content of transgenic rices grown in a closed cultivation system was more stable than that of rices grown in a the field, suggesting that the closed cultivation system is suitable for the production of recombinant miraculin.
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Keyword : miraculin, transgenic rice