NISSAN SCIENCE FOUNDATION
日産科学振興財団シンポジウム

Woody Plants Biotechnology Symposium

Woody Plants Biotechnology Symposium
樹木植物バイオテクノロジー国際シンポジウム

主催―(財)日産科学振興財団／奈良先端科学技術大学院大学
Organiser : Nissan Science Foundation / Nara Institute of Science and Technology

日時―平成21年2月26日(木) 9:30am～5:30pm
Date : 9:30am-5:30pm, Thursday, 26th February, 2009

会場―科学技術館 サイエンスホール
Venue : Science Hall, Science Museum, Tokyo

[問い合わせ先]
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財団法人 日産科学振興財団 NISSAN SCIENCE FOUNDATION　NAIST　振興調整費

NISSAN SCIENCE FOUNDATION
Woody Plants Biotechnology Symposium
Thursday, 26th February, 2009
Science Hall in the Science Museum, Tokyo

The success in sequencing of the Arabidopsis whole genome in 2000 has invited crop and plant sciences into a new era that provides us with new concepts in biology and new biotechnology that were not reached with the previous single-gene sciences. The new era is opened in researches in gene functions in many crops, wild plants and woody plants, the genome of which have not been sequenced, rice genome decoding and molecular-based breeding of rice, and transformation of genes into important crops from organisms phylogenetically far from the crops. Plant biotechnology is now not restricted to experimental model plants, but has been extended to feedstock-producing woody plants, such as eucalyptus, gum trees and *Jatropha*. During the great advances in plant sciences in the last decade, the first success in decoding the whole genome of poplar is surely providing the next decade with information that could not be covered by genome information from model annual plants and will contribute to future biotechnology with woody plants.

The Nissan Science Foundation, NSF, has financially supported researchers working in the fields of environmental sciences. The field of sequestration of environmental CO_2 with aid of solar energy is highlighted and emphasized. The Yokota laboratory of NAIST is engaged in the project research, entitled "Strengthening of the Carbon Dioxide-Adsorbing Capacity of Woody Plants by Improving Their Stress Responses", as a special research project supported by the Foundation.

This Symposium is organized by the laboratory, as part of the project research, to discuss the present and future of plant science and biotechnology of woody plants with invited, distinguished scientists in the fields of genome sciences, biotechnology, and physiology and ecology of these plants.

I hope a successful conclusion of this Symposium in our scientific contribution to construction of the sustainable world.

Nissan Science Foundation (NSF)
Woody Plants Biotechnology Symposium
Chair: Akiho Yokota

NISSAN SCIENCE FOUNDATION
Woody Plants Biotechnology Symposium

Program

9:30- 9:40 **Opening Remarks**

A. Yokota (Nara Institute of Science and Technology)

9:40-10:10 **Plenary Lecture**

A. Shinmyo (Emeritus Professor, Nara Institute of Science and Technology)
Plants save Future of People and Earth

10:10-11:50 **Invited Speakers in Genomics and Molecular Session**
Chaired by Dr. S. G. Maphanyane (Dept. Agric. Res., Botswana)

C. Douglas (University of British Columbia, Vancouver)
The *Populus* Genome: Towards Understanding the Regulation of Secondary Wall Formation and Optimization of Biofuels Traits

D. Shibata (Kazusa DNA Research Institute)
Omics approches for industrial uses of woody plants

T. Demura (RIKEN Plant Science Center)
Molecular Mechanism of Cellulosic Biomass Production

11:50-13:40 **Lunch and Poster Session**

13:40-15:10 **Invited Speakers in Biotechnology**
Chaired by Dr. S. Suharsono (Res. Inst. Biores. Biotechnol., Indonesia)

K. Shinohara (Forestry and Forest Product Research Institute)
Development of Genetically Modified Trees in Japan

T. Kawazu (Oji Paper Co., Ltd.)
***Eucalyptus* Tree Breeding for Improving Productivity in Acid-soil Areas**

T. Hayashi (Kyoto University)
Loosening xyloglucan accelerates the enzymatic hydrolysis of cellulose in wood

15:10-15:40 **Coffee Break and Poster Session**

15:40-17:20 **Invited Speakers in New Directions in Wood Biotechnology**
Chaired by Dr. E. Peloewetse (Dept. Biol., Univ. Botswana)

D. Kramer (Washington State University at Pullman)
Why is Natural Photosynthesis so Inefficiency? How to Cope with Excess Solar Energy in Woods

K. Akashi (Nara Institute of Science and Technology)
Wild plant resources for studying environmental stress tolerance and plant productivity

K. Hikosaka (Tohoku University)
Interspecific variations in photosynthetic capacity: what is different between trees and herbs?

17:20-17:30 **Closing Remarks**

E. Abe (Nissan Science Foundation)

Posters

P-1 **Nitration of peripheral proteins of photosystem II by atmospheric nitrogen dioxide suppresses oxygen evolution in plants**
M. Takahashi[1], J. Shigeto[1], K. Asada[2], A. Sakamoto[1] and H. Morikawa[1]
[1]Depart. Math. & Life Sci., Grad. Sch. Sci., Hiroshima Univ., [2]Fac. Engin., Fukuyama Univ.

P-2 **Analysis of regulatory mechanisms of plant growth in response to light environments**
T. Sakai
RIKEN Plant Science Center

P-3 **Changes in Rubisco content during leaf development in *Eucalyptus globulus***
Y. Suzuki[1], T. Kihara-Doi[2], T. Kawazu[2] and A. Makino[1]
[1]Lab. Plant Environ. Resp., Grad. Sch. of Agric. Sci., Tohoku Univ.
[2]Forest. Res. Inst., Oji Paper Company Ltd.

P-4 **Tree Physiology Research for Increasing the Carbon Sequestration and Photosynthetic Capacity of Forest Ecosystems**
H. Ishii
Grad. Sch. Agric., Kobe Univ.

P-5 **Genetic transformation of the symbiotic nitrogen-fixing bacterium *Frankia***
K. Kucho, K. Kakoi, M. Yamaura, S. Higashi, T. Uchiumi, and M. Abe
Depart. Chem. Biosci., Fac. Sci., Kagoshima Univ.

P-6 **Functional analysis of nuclear-genes for chloroplast development using albino mutants in *Arabidopsis***
R. Motohashi[1], T. Kato[1], M. Hara[1], K. Matsuura[1], S. Itayama[1], Y. Akaike[1],
F. Myouga[2], N. Nagata[3] and K. Shinozaki[2]
[1]Shizuoka University, [2]RIKEN Plant Science Center, [3]Japan Women's University

P-7 **pfkB-type carbohydrate kinase family protein, NARA5, is essential for the massive expressions of plastid-encoded photosynthetic genes in *Arabidopsis thaliana***
T. Ogawa[1], K. Nishimura[1], K. Tomizawa[2], H. Ashida[1] and A. Yokota[1]
[1]Grad. Sch. Biol. Sci., Nara Inst. Sci. Technol., [2]RITE

P-8 **A DEAD-box RNA Helicase RH39 Is Required for Chloroplast 23S rRNA Processing and Essential for Plant Development in *Arabidopsis thaliana***
K. Nishimura, T. Ogawa, H. Ashida and A. Yokota
Grad. Sch. Biol. Sci., Nara Inst. Sci. Technol.

P-9 **The long-term responses of the photosynthetic proton circuit to drought**
K. Kohzuma[1], J. A. Cruz[2], K. Akashi[1], S. Hoshiyasu[1], Y. Munekage[1], A. Yokota[1] and D. M. Kramer[2]
[1]Grad. Sch. Biol. Sci., Nara Inst. Sci. Technol.
[2]Inst. Biol. Chem., Washington State Univ., Pullman

P-10 **Energy conversion efficiency by Photosystem II assembled with variant copies of subunit D1 in themophilic cyanobacterium *Themosynechococcus elongatus***

M. Sugiura[1], F. Rappaport[2] and A. Boussac[3]
[1]Cell-Free Science and Technology Research Center, Ehime University
[2]Institut de Biologie Physico-Chimique, CNRS
[3]iBiTec-S, CEA Saclay

P-11 **Probing photosynthesis in the living plant: What can we learn about the limits of photosynthetic energy conversion efficiency?**

D. M. Kramer
Inst. Biol. Chem., Washington State Univ., Pullman

Lecture Session

Overseas invitees: 30-min talk and 10-min discussion
Japanese invitees: 25-min talk and 5-min discussion

Plants save Future of People and Earth

Atsuhiko Shinmyo
Emeritus Professor, Nara Institute of Science and Technology, Japan

In the 20th century, the world population increased from 1.6 billion to 6 billion as a result of a drastic increase of food production established by using huge amount of petroleum. Present agricultural production in the developed countries is supported by high energy consuming cultivation, such as agricultural machines and implement, irrigation, chemical fertilizer, and chemical herbicides and insecticides. Petroleum brought not only pleasant and convenient life in the developed countries, but also life of 300 - 400 million peoples in a cold district, such as Hokkaido, Canada, northern USA and northern Europe.

It is estimated that the world population of 6.7 billion in 2008 will reach to 9 billion. Is it truth? Petroleum will be exhausted within 40 years. Increase of food production to support 9 billion populations without petroleum will be very difficult. Program of the exchange of gasoline and diesel oil to biofuel, such as ethanol and biodiesel from sucrose, starch and rape seed, suppresses the food supply. Although biofuel production will be changed from biomass competing to foods and feeds to unused cellulosic materials, an efficient conversion of lingo-cellulose to biofuel have to overcome high technological barrier.

It is said that the Japanese forest 100 year ago was very poor, since peoples used woods as fuel, but it has been recovered after start to use petroleum as fuel. Russia stopped to send the natural gas to the Ukraine on Jan. 10, 2009 and it affected the supply to Europe also. TV reported that many European peoples used the woods to survive in the cold winter. The best solution to overcome the shortage of petroleum might be increase of production of plant biomass including cereals, root vegetables, oil plants, and woods.

Annual production of plant biomass energy is 10 times of the present use, of which almost 90% is fossil resources. Plant productivity is significantly suppressed by a lot of environmental stresses. Stress-resistant genes can recover the reduction of plant productivity by using recombinant DNA technology. It is possible to increase the maximum productivity genetically determined. There are huge areas unsuitable for agriculture in the world, such as acidic and alkaline soil, salty and dry land, cold and hot weather. Growth of plants in these areas will be possible by the progress of plant molecular biology and biotechnology.

The *Populus* Genome: Towards Understanding the Regulation of Secondary Wall Formation and Optimization of Biofuels Traits

Carl J. Douglas
Department of Botany, University of British Columbia, Vancouver, BC, Canada

Poplars and aspens (*Populus*) have potential as north-temperate biofuels feedstocks, and possess cell wall biochemistries that are relatively readily converted to fermentable sugars for bioethanol production. Northwestern North America including British Columbia (BC) has a huge genetic diversity in its native *Populus* species, and a focus on *Populus* genetic variants deployed in plantations has many advantages. Despite this potential, many hurdles must be overcome if a *Populus*-based bioenergy sector is to become a reality. A major challenge will be to achieve rapid genetic improvement and domestication of poplars with respect to biofuels and biomass traits. The draft genome of *Populus trichocarpa* (Nisqually-1 genotype) was first released in 2005 and published in 2006. This genome resource has opened the door both to a better understanding of the regulation of wood and secondary wall formation, and to rapid genetic improvement of *Populus* for biofuels applications. We and our collaborators are taking a two-pronged approach towards this goal. First, we are using genomics and comparative genomics to better understand the genes that control secondary cell wall biosynthesis and its regulation. This will help to inform candidate genes that may affect secondary cell traits, and examples of these will be discussed. Secondly, we are targeting the discovery genetic variation, predominantly singe nucleotide polymorphisms (SNPs), in a large suite of such poplar candidate genes, in order test the association of allelic variation in such genes to biofuels and biomass related phenotypes. To achieve the latter goal, we are using a collection of 500 wild *P. trichocarpa* genotypes from BC and the adjacent states of Oregon and Washington, replicated in field trials, to generate a SNP polymorphism database for 7000 candidate genes. In parallel, we will generate wood chemistry and adaptive trait phenotypes for the same genotypes. This will culminate in the determining the SNP genotypes for at least 600 candidate genes in 500 individuals, to test for genotype-phenotype associations. Our initial experiments are aimed at SNP discovery by transcriptome resequencing, using next generation sequencing and the *P. trichocarpa* Nisqually-1 genome sequence as a reference. Initially, eight *P. trichocarpa* individuals from a common population but spanning a geographic range of 54°N to 49°N were selected for xylem transcriptome resequencing. Initial resequencing results from this first set of individuals will be discussed.

Omics approaches for industrial uses of woody plants

Daisuke Shibata
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With a wealth of cDNA and genomic sequences of various plant species including woody plants, my lab has been working on omics technologies, especially those in metabolomics for plant biotechnology. One of the major drawbacks in metabolomics is the difficulty of identifying metabolites in a high-through-put, non-targeted manner. Thus, we introduced a liquid chromatography-coupled Fourier transform ion cyclotron resonance mass spectrometer (LC-FT/ICR-MS) to analyze metabolites with a high mass accuracy of 0.5 ppm. Such ultra-high accuracy allows us to predict a molecular formula or possible formulas of a metabolite, and furthermore, the MS/MS fragmentation patter of the metabolite produced from the machine provides a cue to speculate the chemical structure. We are challenging toward a comprehensive annotation of metabolites in various organisms, including diverse plant species including woody plants, microorganisms and animals, which will provide a firm basis to the metabolomics research. For example, over 800 tomato metabolites including many novel ones were annotated (Iijima *et al., Plant J.* 54, 949-962, 2008). However, the annotation process was very time-consuming. Even for well-trained chemists, a working period of at least three months (!!) to a single tomato sample was needed to process the whole chromatography dataset. Thus, we developed the pipeline PowerFT to mimic the annotation processes in a computational way, where the whole processes of peak detection, isotope peak identification, formula prediction and web-information search were automated within 30 minutes. We also prepared another pipeline, PowerMatch, to compare the processed data sets on a graphical interface. To accommodate the curated metabolite datasets, we are now constructing the database "KOMICS" (Kazusa OMICS, http://webs2.kazusa.or.jp/komics/).

To integrate quantitative datasets of transcripts and metabolites on metabolic maps, we developed a graphical interface KaPPA-View (Tokimatsu *et al., Plant Physiology* 138, 1289-1300, 2005, http://kpv.kazusa.or.jp/kappa-view3/). Some examples of application of our technologies to woody plants will be presented.

This work was partly supported by the Kazusa DNA Research Foundation and New Energy and Industrial Technology Development Organization (NEDO) as part of a project called “Development of Fundamental Technology for Controlling the Material Production Process of Plants”.

Molecular Mechanism of Cellulosic Biomass Production

Taku Demura
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Cellulosic biomass, composed mainly of cellulose, hemicellulose, and lignin, is an attractive renewable energy feedstock to be converted into biofuels such as bioethanol. Since the plant secondary cell walls produced in wood and xylem are the major sources of cellulosic biomass, it is extremely important to unravel the mechanisms of plant cell wall formation. We have been studying regulatory mechanisms of secondary cell wall formation using model cell culture systems in *Zinnia elegans* and *Arabidopsis thaliana* in combination with the comprehensive gene expression profiling and the reverse genetic analysis of candidate genes controlling secondary cell wall formation. Our efforts resulted in the identification of a number of important regulatory genes including several NAC-domain and MYB-related transcription factors.

Arabidopsis VASCULAR-RELATED NAC-DOMAIN6 (VND6) and VND7, encoding NAC-domain transcription factors, can induce secondary cell wall formation of xylem vessels, not only in *Arabidopsis* plants but also in tobacco BY-2 cultured cells, while the loss of function, by overexpression of dominant suppressor forms of VND6 and VND7, inhibits secondary cell wall formation of vessels. Further characterization of VND6 and VND7 and closely related proteins (VND1 to VND5) revealed that the VND proteins cooperatively regulate secondary cell wall formation. We recently found that another NAC-domain transcription factor (VNI2) interacting with VND7 negatively regulates VND7 function. In addition, several R2R3-Myb transcription factors that are expressed specifically in xylem vessels and fibers are found to regulate secondary cell wall formation, of which *Arabidopsis* MYB46 and MYB83 were shown to function redundantly in secondary cell wall formation in vessels. Based on the results we have recently started reverse genetic analysis of these genes in trees. Overexpression of *Arabidopsis* VND6 and VND7 in poplar resulted in the ectopic formation of secondary cell walls in leaves. In contrast, overexpression of dominant suppressor forms of VND6 and VND7 inhibited secondary wall formation of xylem cells to some extent, suggesting that VND6 and VND7 function as positive regulators of secondary cell wall formation in tree species.

Development of Genetically Modified Trees in Japan

Kenji Shinohara
Department of Molecular and Cell Biology,
Forestry and Forest Products Research Institute (FFPRI), Japan

The nucleotide sequence of the entire genome of black cottonwood, *Populus trichocarpa*, was determined in 2006, and at present two research groups in Japan and US is determining that of *Eucalyptus*. However, it is quite difficult to study the structural genomics of *Cryptomeria japonica* D. Don because of its large genome. The FFPRI has succeeded in the large-scale collection of 19,841 nonredundant *P. nigra* full-length enriched cDNA clones by collaboration with the RIKEN Plant Science Center. Population of these cDNA clones represents approximately 44% of the predicted genes in the *Populus* genome. And the FFPRI has also collected 10,464 nonredundant full-length enriched cDNA clones of *C. japonica* male strobili. Full-length cDNA resources are extremely useful, not only for gene annotation and the determination of transcriptional start sites, but also for functional analyses. These will be valuable tools to provide further insight into the genomics of woody plants and transcriptome.

Genetically modified trees are available for the prevention of global warming. I am interested in the growth control of woody plants by genetic engineering with the above cDNA resources. It is possible to genetically engineer trees that grow faster and produce more biomass than the wild type, simply by the down-regulation or the constitutive expression of genes for the biosynthesis of ethylene or gibberellin. One cDNA clone encoding the poplar terminal flower 1 ortholog that acts as a repressor of flowering in vegetative tissues has also been isolated. An early flowering variety of transgenic poplar was generated by the down-regulation of the gene. However, the growth of transgenic poplar was drastically inhibited. Therefore, the suppression of flowering will lead to the enhanced productivity. I also introduce the improvement in environmental stress tolerance by genetic engineering in Japan. There are several stress-tolerant transgenic trees, such as the drought-tolerant *eucalyptuses* and acid-tolerant *eucalyptuses* by the Oji Paper Co., Ltd., the salt-tolerant *eucalyptuses* by the Nippon Paper Group, Inc. and the ozone-tolerant poplars by the FFPRI. I will discuss the availability of genetically modified trees for the prevention of global warming.

Eucalyptus Tree Breeding for Improving Productivity in Acid-soil Areas

Tetsu Kawazu
Forestry Research Institute, Oji Paper Co., Ltd., Japan
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In the pulp and paper industry, raw material and fuel costs are rising and competition is intensifying. As a result, the importance of a resource strategy targeting the stabilization of raw material procurement grows each year. In particular, wood chips are expected to be in short supply worldwide, and Japanese paper-manufacturing companies make efforts to forest overseas. However, overseas forestation faces difficulties in procuring sites for forestation. In addition, due to the global shortage of grain, there is competition for the use of land with agriculture. Under these circumstances, forestation using trees with high growth performance and high wood quality is important. Or else, forestation will expand to the fields with serious environmental problems. Therefore, it is important to produce trees with tolerance to environmental stress such as phytotoxicity in rhizosphere, drought, and poor nutrition by conventional and molecular breeding technology.

Acid soils that limit plant growth are found throughout the world and cover 30-40% of the world's arable soils. A lot of factors contribute to acid soil toxicity depending on soil composition. The primary factor limiting plant growth is aluminum (Al) toxicity. For most plants, exposure to Al causes severe inhibition of root growth. And plants in acid soils also suffer from deficiencies of phosphorus, nitrogen, calcium, magnesium and potassium. The resulting restricted root system has trouble in nutrient and water uptake, making the plants more susceptible to drought stress (Samac and Tesfaye 2003). An effective approach managing acid soils is the application of lime. However, large-scale liming is not practical in forestry due to the costs. In such areas, Al-tolerant (acid soil tolerant) plants are being developed through selection and breeding. Although the exact mechanisms of acid soil tolerance have not been clearly defined yet, intense research efforts have succeeded in identifying the major mechanism of Al-tolerance. Excretion of organic acid, especially citric acid, from roots is an efficient acid soil-tolerant mechanism (Ma *et al*. 2001). There are several successful reports that the modification of citric acid metabolism by the gene manipulation in herbaceous plants could improve plant growth on acid soils probably due to enhancement of tolerance to Al toxicity and acquisition of phosphate from insoluble phosphate compounds (Koyama *et al*. 2000).

Eucalyptus is the most widely planted hardwood tree in the tropical and subtropical areas because of its superior growth, broad adaptability and multipurpose wood properties. To apply the molecular breeding technology to *Eucalyptus* breeding, we developed a unique transformation system of *Eucalyptus* trees using shoot primordium formation (Kawazu *et al*. 2003). We have produced hybrid *Eucalyptus* (*Eucalyptus grandis* x *E. urophylla*) transgenic plants with overexpression of mitochondrial citrate synthase (CS) gene isolated from carrot or stable knock-down of NADP-isocitrate dehydrogenase (ICDH) by the RNAi technique. Both transgenic lines showed increased citrate accumulation and enhancement of citrate excretion from the roots, which showed improved growth on acid soil in greenhouses.

Results and Discussion

Eucalyptus was transformed with the full length CS cDNA from carrot (Takita *et al*. 1999) or the RNAi cassette using 3'-UTR of ICDH from *Eucalyptus*. These genes were controlled by CaMV 35S promoter. We selected two lines each for both transformants by the assay of the enzyme activity. Activities of other enzymes related to citrate metabolism in transformants were almost the same as those of the host

Eucalyptus line. These transgenic lines with higher CS activity or lower ICDH activity increased citrate concentration in roots and enhanced their ability of citrate excretion from roots compared with the host line. Citrate excretion level of transgenics was 1.5-1.7 times higher than that of the host line.

When grown on Japanese acid soil (Andisols), CS-overexpressed *Eucalyptus* line improved its growth, especially root growth, compared with that of the host line. Also, the transgenic line with repressed ICDH expression showed improved growth on the Japanese acid soil, too.

These results indicate that the modification of citric acid metabolism to accumulate more citrate by gene manipulation, i.e. overexpression of CS and/or repression of ICDH is a useful approach to enhance citrate excretion from the roots and in turn improve plant growth on acid soils. However, transgenic tobacco plants with overexpressed CS or repressed ICDH did not show significant increase of citrate concentration in tissue and citrate release, and did not enhance its Al tolerance (Delhaize *et al*. 2003). Therefore, the effect of these approaches might depend on the plant species. In order to evaluate growth performance of transgenic eucalypts on acid soil of the Brazilian commercial plantation, cultivation test of transgenic eucalypts overexpressing CS was conducted. In this experiment, micro-grafted transgenic eucalypts (grafted non-transgenic eucalypts on transgenic roots) showed higher response in P accumulation and greater primary growth under the condition of lower P availability than controls.

Genetic modification of citrate metabolism in eucalypts has some ameliorating effects on enhancing tolerance to acid soil. For further improvement of citrate excretion ability in transgenic plants, it would be needed to co-regulate the ability of citrate transport on plasmamembrane. One of important proteins in citrate efflux is a citrate transporter. Recently, a citrate transporter gene was isolated from barley (Furukawa *et al*. 2007). In the report, introduction of the citrate transporter gene enhanced citrate excretion by four-fold compared with that of control plants and conferred Al tolerance to tobacco. On the basis of this idea, we are promoting the isolation of transporter genes and associated genes from eucalypts. Moreover, we would like to put such transgenic eucalypts to practical use. We have to consider the diffusion of transgenes to environment by pollen. In this project, micro-grafting was employed and was recognized to be one of effective ways. For a general system to prevent the diffusion of pollen, we have tried the development of non-flowering mutants by the heavy ion beam irradiation.

Acknowledgment

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Loosening xyloglucan accelerates the enzymatic hydrolysis of cellulose in wood

Takahisa Hayashi, Rumi Kaida, Tomomi Kaku and Kei'ichi Baba
Kyoto University, RISH, Gokasho, Uji, Kyoto 611-0011, Japan

Cellulase does not easily hydrolyze 1,4-β-glucan intercalated with hemicellulose nor does hemicellulase efficiently attack hemicellulose intercalated tightly into microfibrils in the xylem because plant cell elongation and expansion further tighten the intercalation between 1,4-β-glucans and hemicellulose during growth. The question at the xylem is whether the hydrolysis of cellulose could be enhanced by the prevention of hemicellulose association with its constitutive degradation during growth. Our aim was to assess the intercalation of hemicellulose as a recalcitrance of cell walls to saccharification. Here, we produced several transgenic poplars overexpressing xyloglucanase, cellulase, xylanase, galactanase and polygalacturonase, in hopes of creating useful plants for the production of biofuels. The saccharification and fermentation was higher in the xylem overexpressing xyloglucanase, cellulase and xylanase than in that of the wild-type plant. Treatment with ultrasonication allowed solubilization of xyloglucan and xylan from the xylem of the wild-type poplar and enhanced its saccharification. These results show that the elimination of xyloglucan and xylan promotes saccharification of cellulose microfibrils and leads to an increased ethanol productivity. This is probably because the amorphous regions of cellulose microfibrils are intercalated and cross-linked with each other by these two polysaccharides.

Why is Natural Photosynthesis so Inefficiency? How to Cope with Excess Solar Energy in Woods

David M. Kramer
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This talk will focus on why photosynthesis is not as efficient as is theoretically possible, and what, if anything, we can do about it. These issues are critical for efforts to improve photosynthesis for food production, biofuels production and carbon sequestration. I will argue that natural photosynthesis did not evolve as a maximally efficient energy storage system, nor to make biofuels, but instead evolved as an add-on module to life, allowing organisms to tap into a huge new power source, solar energy, and thus to colonize and survive in new environments far from geochemical energy sources. Photosynthesis is thus limited by what I call 'legacy biochemistry', and under the conditions that really matter for bioenergy, plants are overpowered by solar energy. Regulating this energy is critical for avoiding deleterious side reactions (photodamage). Consequently, natural photosynthesis is 'tuned' both by the requirements of sustaining, while not hindering, the processes of life. To improve photosynthetic efficiency, it is essential to understand how the light reactions are integrated into the rest of plant biochemistry. The talk will cover areas where integration is finely tuned in surprising ways, with important lessons for potential engineering efforts.

Wild plant resources for studying environmental stress tolerance and plant productivity

Kinya Akashi

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Earth's land accommodates estimated ~250,000 terrestrial plant species, which are extremely diversified in their life form, morphology and physiology. Distribution of the diversified plants in various ecological niches must have been accompanied with their effective acclimation mechanisms to the habitats. Water deficits are recognized as the prime factor limiting vegetative growth and ecological distribution of terrestrial plants. Although a small group of plants termed xerophytes are unique for their tolerance to drought in the presence of strong light, little has been known for the molecular mechanisms underlying their successful adaptation to the arid environments. Wild watermelon (*Citrullus lanatus*) is a C_3 xerophyte native to the Kalahari Desert, and has been studied as the model xerophyte to understand how C_3 plants can survive under harsh environmental stresses. A number of desirable physiological traits have been recognized in this plant, such as vigorous root growth under drought, maintenance of leaf water status and tolerance to excess light conditions. Transcriptome and proteome analyses have highlighted many useful genes, proteins and metabolites, which offer a wide range of possibilities for improving environmental stress tolerance and biomass productivity of other plants through molecular breeding. A promising target for the breeding program is *Jatropha curcus*, which efficiently produces oil-rich seeds suitable for biodiesel, and possesses great potentials for biofuel production in semi-arid areas in the world. We launched the research and development program for *Jatropha* through the international consortium, which aims at genetic improvement and field experiments of this plant in Southeast Asia and African continent. The perspectives and current status of this project is also presented in this talk.

Interspecific variations in photosynthetic capacity : what is different between trees and herbs?

Kouki Hikosaka
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Photosynthetic capacity is known to vary considerably among species. It is well known that woody species tend to have lower photosynthetic capacity than herbaceous species. Its physiological cause and ecological significance have been one of the most fundamental questions in plant ecophysiology. Photosynthetic nitrogen-use efficiency (PNUE, photosynthetic rate per unit leaf nitrogen) has attracted much attention to understand the inherent variation in photosynthetic capacity among species. Previous studies have demonstrated that leaf nitrogen is less allocated to Rubisco in low-PNUE species. It was considered that there is a trade-off in nitrogen partitioning between photosynthesis and cell walls. We studied contents of Rubisco (key enzyme of photosynthesis) and cell walls in leaves of 26 species with a large variation in photosynthetic rates, which covered almost half of its global variation. Our results demonstrated that Rubisco-use efficiency (RUE, photosynthetic rate per Rubisco) explained most of the interspecific variation in photosynthetic rates, while nitrogen allocation to Rubisco had a significant but smaller contribution. Our analyses suggested that RUE was altered by the content of cell wall, which probably affected mesophyll conductance for CO_2 diffusion. Cell wall nitrogen increased with increasing leaf mass per area, but it did not directly affect the variation in photosynthetic rates. Species with low photosynthetic rates invest more resources in cell walls at the expense of RUE.

Poster Session

P1

Nitration of peripheral proteins of photosystem II by atmospheric nitrogen dioxide suppresses oxygen evolution in plants

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Nitrogen dioxide (NO_2) has been known as an air pollutant that suppresses the photosynthesis in plants. However, the molecular mechanism is still unclear. In this communication, we investigated by means of antibody-based proteomics nitration of proteins in *Arabidopsis thaliana* leaves that were fumigated with 4-40 ppm NO_2. We found that immunoreactive protein spots were identified almost exclusively to be PsbO or PsbP, peripheral proteins of the oxygen evolving complex (OEC) of PSII. The same was true when isolated thylakoid membranes were treated with NO_2. A positive linear relationship was observed between NO_2-induced nitration of PsbO/PsbP and NO_2-mediated inhibition of oxygen evolution in isolated thylakoid membranes. The nitration of PsbO/PsbP by NO_2 was found to be accelerated by light and reactive oxygen. We therefore hypothesize that nitration of tyrosine residues of PsbO/PsbP proteins in the OEC is involved in the inhibition of photosynthetic oxygen evolution by stresses such as reactive nitrogen, reactive oxygen and light. Furthermore, the western blot analysis using the anti-3-nitrotyrosine antibody of *Daphniphyllum macropodum*, a woody plant species, also strongly suggested that its PsbO/PsbP proteins were nitrated upon fumigation with NO_2.

P2

Analysis of regulatory mechanisms of plant growth in response to light environments

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Plants regulate their growth directions in response to light direction to optimize light capture for photosynthesis, and its response is called phototropism. In higher plants, phototropism is explained by the Cholodny and Went hypothesis, which proposes that the asymmetric distribution of the phytohormone auxin causes the differential growth and consequent bending of plant organs, and three major families of photoreceptors, phototropins, phytochromes, and cryptochromes, coordinately regulate its response. We are studying on these molecular mechanisms by a molecular genetic approach using *Arabidopsis thaliana* mutants, and identified several signaling factors in this response. We identified a novel blue-light photoreceptor phot2, and indicated that phot1 and phot2 show partially overlapping functions in the phototropic response in a fluence rate-dependent manner. Phototropin signaling probably controls the auxin transport activities to generate the asymmetric distribution of auxin, and we are now studying about its molecular mechanisms. In addition, our current results indicated that *Arabidopsis* hypocotyls contain not only phototropism-inducing mechanisms but also phototropism-suppressing mechanisms, and that photoreceptors, phytochromes and cryptochromes enhance the phototropic response by removing suppressors of the phototropic response. One of the phototropism-suppressing mechanisms was found to be mediated through the ABC-type auxin transporter ABCB19, and activations of phytochromes and cryptochromes suppress the ABCB19 expression and enhance the phototropic response indirectly. In this symposium, I introduce our results and new finding in this field.

P3

Changes in Rubisco content during leaf development in *Eucalyptus globulus*

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Rubisco (EC. 4. 1. 1. 39) is a key enzyme in photosynthesis and the most abundant leaf protein in higher plants and changes in its content during leaf development is important in both the C and N economy of the plant. In grass plants, Rubisco content increases during leaf expansion, becomes maximum at maturation and gradually declines during senescence. In rice and soybean, Rubisco synthesis is active before leaf maturation but rapidly declines thereafter, whereas its degradation becomes active (1,2). Thus, changes in Rubisco content are a result of dramatic changes in balance of protein turnover. In woody plants, on the other hand, information on Rubisco turnover is not available. In this study, changes in Rubisco content and it synthesis were examined in *Eucalyptus globulus* seedlings. Plants were hydroponically grown in a greenhouse. All axillary buds were removed just after their emergence and difference in leaf position approximately reflected that in leaf age by this treatment. At 4.5 months after sowing, the plants were ^{15}N-labeled then leaves at different positions are collected. The amount of Rubisco per unit fresh weight slightly increased with leaf expansion, became maximal in uppermost fully expanded leaves, was kept constant in leaves at middle position, then declined in lower leaves. The amount of Rubisco synthesized per unit fresh weight was maximal in the expanding leaves, rapidly declined and became quite low after full expansion. Rubisco was scarcely synthesized in leaves at middle and bottom positions. Therefore, it is suggested in *Eucalyptus globulus* that an increase in Rubisco content in expanding leaves was accounted for by its active synthesis, whereas Rubisco content was kept constant by slow protein turnover after leaf maturation and Rubisco degradation became active in late stage of leaf development. This pattern is basically the same as in rice and soybean.

References

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(2) Tonouchi A, Makino A, Mae T, Ohira K (1988) Jpn J Soil Sci Plant Nutr 59, 573

P4

Tree Physiology Research for Increasing the Carbon Sequestration and Photosynthetic Capacity of Forest Ecosystems

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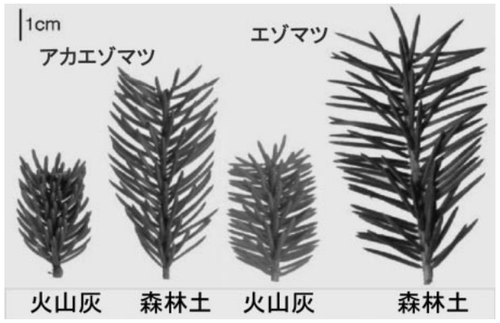
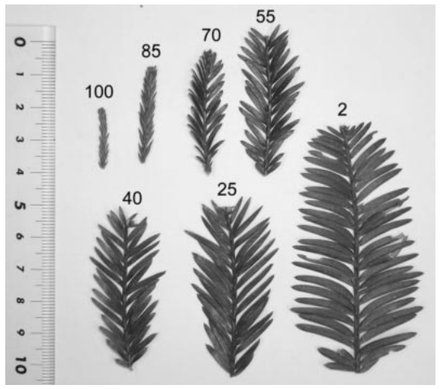
Of the various terrestrial ecosystems, forests hold the largest carbon store. Atmospheric CO_2 concentration could potentially be reduced by accelerating carbon uptake by forest ecosystems. Development of new tree varieties with high carbon sequestration capabilities could potentially increase carbon uptake through reforestation. We have identified several phenotypic characteristics that could potentially increase tree photosynthesis. Plasticity of needle morphology and their arrangement on twigs, as well as the allocation of chlorophyll and other enzymes within the crown are important characteristics that allow trees to capture light efficiently. The present study aims to apply these findings to develop tree varieties with high photosynthetic capacities. Our goal is to elucidate the contribution of morphological and allocational plasticity toward increasing photosynthetic capacity and to elucidate the quantitative genes that determine these traits.



Morphological changes of *Picea glehnii* (left) and *Picea jezoensis* (right) planted on nutrient poor volcanic ash (short shoots) and nutrient rich forest soil (long shoots). Such changes have consequences for photosynthetic rate and stand productivity.



Morphological changes observed along the vertical gradient in the canopy of a 100m-tall *Sequoia sempervirens* tree. Such changes have consequences for photosynthetic rate per light capturing area.

Ishii H, Jennings GM, Sillett SC, Koch GW (2008) Hydrostatic constraints on morphological exploitation of light in tall *Sequoia sempervirens* trees. Oecologia 156:751-763
Ishii H, Kitaoka S, Fujisaki T, Maruyama Y, Koike T (2007) Plasticity of shoot and needle morphology and photosynthesis of two *Picea* species with different site preferences in northern Japan. Tree Physiology 27:1595-1605
Ishii H, Ooishi M, Maruyama Y, Koike T (2003) Acclimation of shoot and foliage morphology and photosynthesis of two *Picea* species to differences in soil nutrient availability. Tree Physiology 23:453-461

P5

Genetic transformation of the symbiotic nitrogen-fixing bacterium *Frankia*

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Nitrogen is an essential element for living organisms. In plant, nitrogen plays an important role as a constituent of chlorophyll. Therefore nitrogen supply from soil is a critical factor delimiting the efficient growth of plants. Actinorhizal plants such as *Alnus* and *Casuarina* establish symbiosis in root nodules with a nitrogen-fixing actinobacterium *Frankia* and utilize N_2 gas in atmosphere as a nitrogen source. Actinorhizal plants are mostly woods and grow rapidly even under nitrogen-limited conditions. However, molecular basis of the symbiosis is largely unknown because genetic manipulation of *Frankia* has not been feasible. In this study, using novel technical attempts, we succeeded in transforming *Frankia* sp. strain CcI3 which make symbiosis with *Casuarina* species. We generated fusion marker genes consisting of a tetracycline resistance gene with a high codon usage similarity to *Frankia*'s and promoters of the strain's translation initiation factor 3 gene. We flanked the fusion genes with 4-kb genomic sequences from strain CcI3 in the expectation that they would be integrated into the targeted site by homologous recombination. We introduced the transformation constructs into *Frankia* cells by electroporation and selected transformants in liquid media. The growth of antibiotic resistant cells was observed depending on the presence of construct DNA. Using genomic PCR and reverse transcriptase-PCR analysis, we confirmed that the marker genes had been introduced into the *Frankia* cells. Integration of the marker genes into the chromosome by homologous recombination did occur, but at a low frequency. When we cultured the transformants in fresh selection media, the proportion of the cells containing marker genes was reduced, suggesting that the cultures had generated spontaneous antibiotic resistant mutants. Although several aspects of our procedure need to be improved, we believe that the results mark substantial progress in *Frankia* genetics.

P6

Functional analysis of nuclear-genes for chloroplast development using albino mutants in *Arabidopsis*

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To determine essential nuclear-genes involved in chloroplast development, we systematically analyzed *albino or pale green* mutants in *Arabidopsis*. We have isolated about 40 mutants with *albino or pale green* (*apg*) phenotypes. Identified *APG* genes have sequence homology with house keeping proteins involved in photosynthesis, translation, transcription, translocation and so on. In this symposium, we present phenotypes of six *apg* mutants (*apg4, apg9, apg11, apg12, apg14* and *apg15*), and discuss functions of these *APG* genes in chloroplast development.

The *apg4* mutant has a disrupted gene for a ribosome binding factor RBFA homologue. The phenotypes of the *apg4* mutant had white cotyledons and yellow or green variegated true leaves. In *Escherichia coli*, the RBFA was shown to be involved in processing of the pre-rRNA to form the mature 16S rRNA. We found that the *APG4* is involved in processing of the pre-rRNA to form the mature 23S and 4.5S rRNA and related accumulation of *rrn*16 transcripts. The effect on the *apg4* mutation gradually decreased as plant grew, which may be due to high expression of the *APG4* gene in cotyledon but not in true leaves. We predicted that other proteins function as *APG4* in true leaves. It was reported that RimM (21-kDa protein formerly called 21K) and Era (*E. coli* Ras-like) have similar functions of RBFA in *E. coli*. To analyze the relation between *APG4* and these genes, we identified their homologous genes in *Arabidopsis thaliana*. We report the function and expression of these genes closely related to *APG4*.

APG9, APG14 and *APG15* have DNA or RNA binding domains. We found that mRNA of chloroplast encoded genes have processing defect in these mutants. We report that these proteins related in processing and stability of chloroplast transcripts, too.

P7

pfkB-type carbohydrate kinase family protein, NARA5, is essential for the massive expressions of plastid-encoded photosynthetic genes in *Arabidopsis thaliana*

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The massive accumulation of photosynthetic proteins in chloroplasts depends on many nucleus-encoded factors in higher plants. In order to identify genes involved in the massive accumulation of photosynthetic proteins including RuBisCO, we have isolated the twelve Arabidopsis EMS mutants that show low RuBisCO amounts. These were named *nara* (*genes necessary for the achievement of RuBisCO accumulation*) mutants. Among them, *nara5-1* showed a markedly decrease in plastid-encoded photosynthetic proteins including RuBisCO. Map-based cloning revealed that *NARA5* encodes a chloroplast pfkB-type carbohydrate kinase family protein of unknown function. Analysis of photosynthetic gene expressions during light-induced greening of etiolated seedlings in *nara5-1* and T-DNA insertion mutant, *nara5-2*, indicates that *NARA5* is essential for the massive expressions of plastid-encoded photosynthetic genes, particularly in *rbcL*.

P8

A DEAD-box RNA Helicase RH39 Is Required for Chloroplast 23S rRNA Processing and Essential for Plant Development in *Arabidopsis thaliana*

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In recent years, chloroplasts have attracted attention as a place to synthesize large amounts of foreign proteins because of their high potential for protein synthesis. However, the molecular mechanisms for chloroplast protein synthesis have not been fully understood in higher plants. To gain insight into the mechanism of chloroplast protein synthesis, we have characterized the *nara12-1* (*the gene necessary for the achievement of RuBisCO accumulation*) mutant of Arabidopsis with impairment of the chloroplast translation. Positional cloning of *NARA12* identified a DEAD-box protein RH39. Transient expression of the N-terminus of the RH39-GFP fusion protein in tobacco leaves suggested that RH39 was localized in chloroplasts. Northern blot analysis revealed that chloroplast 23S rRNA processing was defective in *nara12-1*. *RH39* null allele *nara12-2* was embryonic lethal due to abnormal seed formation. Our results suggest that RH39 is essential for chloroplast rRNA maturation and plant development.

P9

The long-term responses of the photosynthetic proton circuit to drought

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Proton motive force (*pmf*) across thylakoid membranes is of crucial importance, not only for harnessing solar energy for photosynthetic CO_2 fixation, but also for triggering feedback regulation of photosystem II antenna. The mechanisms for balancing these two roles of the proton circuit under the long-term environmental stress, such as prolonged drought, have been poorly understood. In this study, we report on the response of wild watermelon thylakoid 'proton circuit' to drought stress using both *in vivo* spectroscopy and molecular analyses of the representative photosynthetic components. While drought stress led to enhanced proton flux via a ~34% increase in cyclic electron flow around PS I, an observed ~5-fold decrease in proton conductivity, g_{H^+}, across thylakoid membranes suggested that decreased ATP synthase activity was the major factor for sustaining elevated q_E. Western blotting analyses revealed that ATP synthase content decreased significantly, suggesting that quantitative control of the complex plays a pivotal role in down-regulation of g_{H^+}. The expression level of cytochrome b_6f complex —another key control point in photosynthesis— also declined, probably to prevent excess-reduction of PS I electron acceptors. We conclude that plant acclimation to long-term environmental stress involves global changes in the photosynthetic proton circuit, in which ATP synthase represents the major key control point for regulating the relationship between electron transfer and *pmf*.

P10

Energy conversion efficiency by Photosystem II assembled with variant copies of subunit D1 in themophilic cyanobacterium *Themosynechococcus elongates*

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The world is more and more confronted to energetic problems. In nature, at the heart of Photosystem II (PSII) is the reaction centre where light energy is quite efficiently converted into electrochemical potential energy and where the water-splitting reaction occurs. In so doing, it releases dioxygen into the atmosphere and provides the reducing equivalents required for the conversion of CO_2 into the organic molecules of life.

PSII complex is a membrane-spanning complex that is constituted of at least 20 subunits, 17 small polypeptides and three extrinsic proteins. The reaction centre consists of two homologous proteins known as D1 and D2 which have five transmembrane a-helicies each. The active site for water oxidation in PSII goes through five sequential oxidation states (S_0 to S_4) before O_2 is evolved. It consists of a Mn_4Ca-cluster which metal ions ligate to amino acid residues of D1 and D2 polypeptides of PSII complex.

Thermophylic cyanobacterium *Themosynechococcus elongatus* has higher oxygen water oxidation activity than the other oxygenic photosynthetic organisms. The genome of *T. elongatus* possesses three variant copies of D1 genes (*psbA*$_1$ - *psbA*$_3$), which the amino acid sequences are not identical although important amino acid residues for photosynthetic function such as electron transfer and ligand for co-factors. In this study, we examined about properties of PSII which is composed of variant copies of D1 including energy conversion efficiency, water oxidation function, and response to stress conditions.

Under usual environmental cultivation conditions, *psbA*$_1$ constitutively expressed. When *T. elongatus* cells were exposed to strong light and low temperature, expression of *psbA*$_3$ was induced. Water oxidation in PSII composed of $PsbA_3$, genetically knocked out *psbA*$_1$ and *psbA*$_2$, was 1.7 times faster than in PSII composed of $PsbA_1$. In thermodynamic properties, the potential difference between S_3 state (donor side) and plastoquinone Q_B^- (acceptor side) in PSII-$PsbA_3$ was smaller than PSII-$PsbA_1$. And the redox potential of Q_A^- was strongly modified. These results suggest that the structural modification of PsbA around acceptor side controls energy conversion efficiency by PSII.

P11

Probing photosynthesis in the living plant: What can we learn about the limits of photosynthetic energy conversion efficiency?

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This presentation will cover recent developments in the non-invasive measurement of photosynthetic processes under steady-state conditions. The light reactions of photosynthesis are replete with species which respond to changes in physiological state. Many of these species can be probed using non-invasive techniques, allowing us to investigate the inner workings of plants as they function in living plants. I will present an integrated review of the types of measurements available that allow us to probe light absorption, electron transfer, proton transfer, ATP synthesis, regulation of antenna function, photodamage and it repair.