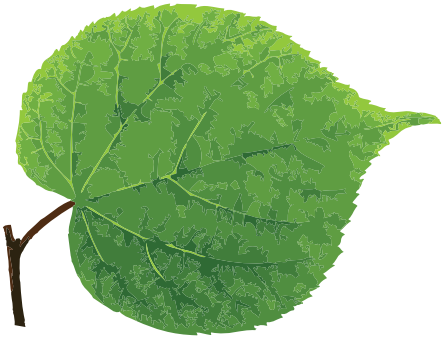

8th International Conference

Photosynthesis and Hydrogen Energy Research for Sustainability

in honor of Agepati S. Raghavendra, William A. Cramer, and Govindjee

October 30 – November 4, 2017
Hyderabad, India

ABSTRACTS AND PROGRAMME

School of Life Sciences
University of Hyderabad

8th International Conference

"Photosynthesis and Hydrogen Energy Research for Sustainability-2017"

in honor of Agepati S. Raghavendra, William A. Cramer, and Govindjee

October 30 – November 4, 2017
Hyderabad, India

Abstracts and Programme

Hyderabad – 2017

8th International Conference "Photosynthesis and Hydrogen Energy Research for Sustainability-2017" in honor of Agepati S. Raghavendra, William A. Cramer, and Govindjee"
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This book contains the abstracts of the lectures and poster presentations at the 8th International Conference on "Photosynthesis and Hydrogen Energy Research for Sustainability-2017: in honor of Agepati S. Raghavendra, William A. Cramer, and Govindjee," held from October 30 through November 3, 2017 at the University of Hyderabad (Department of Plant Sciences, School of Life Sciences), Hyderabad. Both the experimental and theoretical aspects of Photosynthesis and Bio-hydrogen production are covered. Topics range from the primary process of electron transfer and energy bioconversion to the physiology of photosynthesis, as well as the applied aspects of hydrogen production. Special attention is given to discussion of the structural organization of photosynthetic reaction centers, abiotics stress effects on photosynthesis, and mechanisms of hydrogen production. We expect the content of this publication to be of broad interest to all researchers, teachers, and students interested in photosynthesis and/or bio-hydrogen production.

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Additional information is available on our website:
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WELCOME!

We extend a warm and hearty welcome to all the participants of the 8th International Conference on Photosynthesis and Hydrogen Energy Research for Sustainability-2017. This conference is being held during October 30 – November 03, 2017, in the School of Life Sciences, University of Hyderabad, Hyderabad, India. We are proud to tell you that the University of Hyderabad is one of the top Universities in the country, with an outstanding academic ranking. The main purpose of this Conference is to disseminate knowledge in the area of photosynthesis. In addition, we will be honoring three distinguished scientists Professors Agepati S. Raghavendra, William A. Cramer, and Govindjee, who have made pioneering contributions to the field of photosynthesis.

This meeting will be a great occasion to discuss previous, present, and future research on Photosynthesis and Hydrogen Energy, ranging from molecular to global aspects. The Conference has an exciting scientific program covering the areas of Photosynthesis as well as Hydrogen Energy. This meeting will provide a forum for students, postdoctoral fellows, and scientists from different countries to deepen their knowledge and understanding. The Conference will also provide an excellent opportunity for all of us to meet researchers from around the world, widen professional contacts, create new opportunities, and establish new collaborations.

The topics of this conference range widely; they are grouped into two main areas: photosynthesis and biohydrogen. The photosynthesis section includes its primary processes, structure & function of the photosystems, water oxidation mechanism, excitation energy transfer and trapping in the photosystems, generation of the proton electrochemical gradient, as well as biogenesis of the photosynthetic apparatus. Also included are carbon fixation (C3 and C4), photorespiration, artificial and applied aspects of photosynthesis, regulation of photosynthesis by environmental stress and climate change. Additional topics covered are bacterial photosynthesis and its metabolism, systems biology of photosynthesis based on omics approach, photosynthesis education, and emerging techniques for studying photosynthesis. The bio-hydrogen section covers topics of hydrogen energy for the future, hydrogen economy, biological hydrogen production, hydrogenases, proton reduction catalysts, and emerging techniques for studying of hydrogen energy.

We have received tremendous response from India as well as from the overseas; this can be seen in the number of lectures (above 50) and poster presentations (above 120) at this Conference. The Indian organizers have made their best efforts to make this event a grand success and your stay a pleasant and memorable one. We hope you will enjoy the warmth of the University of Hyderabad, the people of Hyderabad, and the world famous Hyderabadi cuisine.

James Barber
Appa Rao Podile
Reddanna Pallu
Venkata Raman Chintalapati
Suleyman I. Allakhverdiev
Rajagopal Subramanyam

Schedule: Photosynthesis Research for Sustainability-2017

*All lectures are invited.

OCTOBER 29 (SUNDAY)

Arrival and Accommodation
Pre-registration at School of Life Sciences
University of Hyderabad

OCTOBER 30 (MONDAY – 1ST DAY)

8:30–9:30 Registration

9:30–9:50 Inaugural ceremony

9:50–10:30 Felicitation to Agepati S Raghavendra, William Cramer & Govindjee. Felicitated by University of Hyderabad

Session 1

Chairpersons: P. V. Sane (India), Suleyman I. Allakhverdiev (Russia), Govindjee (USA)

10:30–11:15 **Johannes Messinger** *(Department of Chemistry, Chemistry Biology Center, Umea University, Umea, Sweden)* From natural to artificial photosynthesis

11:15–11:35 Coffee Break (20 Minutes)

Session 2

Chairpersons: P. V. Sane (India), Johannes Messinger (Sweden), Suleyman I. Allakhverdiev (Russia)

11:35–12:05 **Julian J. Eaton-Rye** *(Department of Biochemistry, University of Otago, New Zealand)* Govindjee and Photosynthesis

12:05–12:35 **Govindjee** *(University of Illinois at Urbana-Champaign, Urbana, USA)* A personal story about Photosynthesis

12:35–13:00 **Marc M. Nowaczyk** *(Plant Biochemistry and Analytical Chemistry, Ruhr University, Bochum, Germany)* Analysis of photosystem II electron transfer by redox polymer/protein biophotoelectrochemistry

13:00–14:00 Lunch

Session 3

Chairpersons: Tatsuya Tomo (Japan), Marc Nowaczyk (Germany), Raimund Fromme (USA)

14:00–14:30 **Rachna Agarwal** *(Nuclear Agriculture and Biotechnology Division; Molecular Biology Division, Bhabha Atomic Research Centre, Trombay, Mumbai, India)* The journey through the structure-function of the complex "cytochrome b_6f": Personal perspective dedicated to Prof. William A. Cramer

14:30–15:00 **William Cramer** (Department of Biological Sciences, Purdue University, West Lafayette, IN, USA) Ironies in photosynthetic electron transport: the cytochrome b_6f lipoprotein complex

15:00–15:30 **Danas Baniulis** *(Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry, Babtai, Kaunas reg., Lithuania / Department of Biological Sciences, Hockmeyer Building of Structural Biology, Purdue University, USA)* Enhanced superoxide production in cytochrome b_6f complex of oxygenic photosynthesis and its role in plant physiology

15:30–16:00 **Barry D. Bruce** *(Biochemistry & Cellular and Molecular Biology Dept., Bredesen Center for Interdisciplinary Research and Graduate Education, University of Tennessee, Knoxville, TN, USA)* The evolutionary pressures for oligomerization

16:00–16:30 **Baishnab C. Tripathy** *(School of Life Sciences, Jawaharlal Nehru University, New Delhi, India)* Towards C4 rice: overexpression of phosphoenolpyruvate carboxylase, phosphoenolpuruvate carboxykinase and carbonic anhydrase in *Arabidopsis thaliana* enhances its photosynthesis, productivity and water use efficiency

16:30–16:45 Coffee Break (15 Minutes)

Session 4

Chairpersons: Yuichiro Takahashi (Japan), Barry D. Bruce (USA), A. N. Mishra (India)

16:45–17:10 **Yuki Kato** *(Graduate School of Science, Nagoya University, Nagoya, Japan)* FTIR study on the redox property of the primary quinone Q_A in photosystem II

17:10–17:35 **Arvi Freiberg** *(Institute of Physics, University of Tartu, Estonia; Institute of Molecular and Cell Biology, University of Tartu, Estonia)* Understanding *in situ* light-harvesting strategies

17:35–18:00 **Kostas Stamatakis** *(Institute of Biosciences and Applications, NCSR "Demokritos", Aghia Paraskevi Attikis, Greece)* The Ross Sea haptophyte *Phaeocystis antarctica* and dinoflagellate cells hosting kleptoplasts derived from it are both capable of light state transitions

18:00–18:20 **Ashwani Kumar** *(Institute of Plant Nutrition, Interdisciplinary Research Center, Justus Liebig University, Giessen, Germany)* Does the first phase of salt stress affect the osmotic and photosynthetic enzyme systems? A review

18:20–20:00 Poster viewing

Chairpersons: Barry Bruce (USA), Marian Brestic (Slovakia), Marc Nowaczyk (Germany), Raimund Fromme (USA), Iwane Suzuki (Japan), Seiji Akimoto (Japan), Cosmin Sicora (Romania), Kostas Stamatakis (Greece), Vasiliy Goltsev (Bulgaria), Marek Živčák (Slovakia), Anjana Jajoo (India), Rajagopal Subramanyam (India), Tripathy B.C. (India), Tatsuya Tomo (Japan), Venkata Mohan S. (India)

20:00 Dinner

OCTOBER 31 (TUESDAY – 2ND DAY)

Session 1

Chairpersons: A. K. Tripathi (India), Santanu Dasgupta (India)

9:00–9:30 **Nathan Nelson** *(Department of Biochemistry, The George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel)* High-resolution structures of plant and cyanobacterial photosystem I

9:30–10:00 **Padmasree K.** *(Department of Biotechnology)* **and Saradadevi Tetali** *(Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad)* Importance of dark respiration in optimizing photosynthetic performance of plants – A turn of the role from in-significance to significance: A personal perspective dedicated to Prof. A. S. Raghavendra

10:00–10:30 **Julian J. Eaton-Rye** *(Department of Biochemistry, University of Otago, New Zealand)* Targeted mutation of D1 and D2 amino acids residues associated with bicarbonate binding and the protonation of plastoquinone B

10:30–11:00 **Ajit V. Sapre** *(Reliance Industries, Mumbai, India)* Biology and engineering innovations to impact photosynthesis and algal productivity

11:00–11:15 Coffee Break (15 minutes)

Session 2

Chairpersons: Keisuke Takagi (Japan), Yuki Kato (Japan), Basanti Biswal (India)

11:15–11:45 **Shree Kumar Apte** *(Emeritus Professor-HBNI, J C Bose National Fellow-DST, Raja Ramanna Fellow-DAE, Bhabha Atomic Research Centre, Mumbai, India)* Photosynthesis and nitrogen fixation (Photodiazotrophy) under stress: cyanobacterial remedies

11:45–12:15 **Agepati S. Raghavendra** *(Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad, India)* Cross-talk of chloroplasts with mitochondria and peroxisomes: Mitochondrial redox is a major signal to mediate the interactions

12:15–12:40 **Shinji Masuda** *(Center for Biological Resources & Informatics, Tokyo Institute of Technology, Japan)* Identification and characterization of a novel chloroplast protein controlling non-photochemical quenching under fluctuating light

12:40–13:05 **Vinzenz Bayro-Kaiser** *(Tel Aviv University, Tel Aviv, Israel)* Temperature-sensitive PSII: A novel approach for sustainable photosynthetic hydrogen production

13:05–14:00 Lunch

Session 3

Chairpersons: Shinji Masuda (Japan), Julian Eaton-Rye (New Zealand), Agepati S. Raghavendra (India)

14:00–14:30 **Yuichiro Takahashi** *(Research Institute for Interdisciplinary Science, Okayama University, Japan)* Identification and characterization of a photosystem I assembly apparatus

14:30–15:00 **Sanjay Kumar** *(Director of CSIR-IHBT, Palampur, India)* Is photosynthetic behavior of plants different at high altitude?

15:00–15:30 **Daisuke Takagi** *(Department of Biological and Environmental Science, Graduate School of Agricultural Science, Kobe University, Kobe, Japan)* Chloroplastic ATP synthase modulates H^+-gradient across the thylakoid membranes for preventing Photosystem I photoinhibition in higher plants

15:30–16:00 **Iwane Suzuki** *(Graduate School of Life Environmental Science, University of Tsukuba, Tsukuba, Japan)* Modification of cyanobacteria for the produce useful compounds

16:00–16:20 Coffee break (20 minutes)

Session 4

Chairpersons: Sergey Shabala (Australia), L. C. Rai (India)

16:20–16:45 **Attipalli R. Reddy** *(Department of Plant sciences, University of Hyderabad,Hyderabad, India)* Carbon flow into lipids: A regulatory mechanism in seed oil biosynthesis in biofuel tree species

16:45–17:10 **Oula Ghannoum** *(ARC Center of Excellence for Translational Photosynthesis; Hawkesbury Institute for the Environment, Western Sydney University, Australia)* Acclimation of C4 photosynthesis to low light

17:10–17:30 **Rasineni Girish Kumar** *(Sandor Life Sciences Pvt. Ltd., Hyderabad, India; Department of Biochemistry, University of Nebraska, Lincoln, USA)* Posttranslational modifications in *Chlamydomonas* Rubisco influence catalysis

17:30–17:45 **Sai Kiran Madireddi** *(Department of Plants Sciences, School of Life Sciences, University of Hyderabad, Hyderabad, India)* LHCSR3 impairs photosynthetic membrane complex assembly of *Chlamydomonas reinhardtii* under drought stress

17:45–19:10 Poster viewing/discussion

Chairpersons: Barry Bruce (USA), Marian Brestic (Slovakia), Marc Nowaczyk (Germany), Raimund Fromme (USA), Iwane Suzuki (Japan), Seiji Akimoto (Japan), Kostas Stamatakis (Greece), Vasiliy Goltsev (Bulgaria), Cosmin Sicora (Romania), Marek Živčák (Slovakia), Anjana Jajoo (India), Rajagopal Subramanyam (India), Tripathy B.C. (India), Tatsuya Tomo (Japan), Venkata Mohan S. (India)

19:10–20:40 Cultural Program

Classical and folk dance performance representing the tradition and diversity of India

20:40 Dinner

NOVEMBER 1 (WEDNESDAY – 3RD DAY)

Session 1

Chairpersons: William Cramer (USA), Iwane Suzuki (Japan), Rajagopal Subramanyam (India)

9:00–9:30 **Sergey Shabala** *(School of Land and Food, University of Tasmania, Australia)* Membrane transport in chloroplasts: optimising cell performance for adverse environmental conditions

9:30–10:00 **Raimund Fromme** *(Arizona State University, School of Molecular Sciences, Tempe; Center of Applied Structural Discovery, Biodesign Institute, Tempe, USA)* Structure of the symmetric photosystem from *Heliobacterium modesticaldum*

10:00–10:30 **Suleyman I. Allakhverdiev** *(Controlled Photobiosynthesis Laboratory, Institute of Plant Physiology, RAS, Moscow, Russia; Institute of Basic Biological Problems, RAS, Pushchino, Russia; Department of Plant Physiology, Faculty of Biology, M.V. Lomonosov Moscow State University, Moscow, Russia; Moscow Institute of Physics and Technology, Dolgoprudnaya, Moscow Region, Russia; Bionanotechnology Laboratory, Institute of Molecular Biology and Biotechnology, Azerbaijan National Academy of Sciences, Baku, Azerbaijan)* A set-up for studying effects of environmental factors on a photocurrent generated by a solar cell based on titanium dioxide and plant photosensitizers

10:30–11:00 **Robert Fluhr** *(Department of Plant and Environmental Sciences, Weizmann Institute, Rehovot, Israel)* Singlet oxygen stress induces arrest of cellular translation

11:00–11:20 Coffee Break (20 minutes)

Session 2

Chairpersons: Kentaro Ifuku (Japan), Sanjay Kumar (India), Agapati S. Raghavendra (India), P. B. Kirti (India)

11:20–11:50 **Olaf Kruse** *(Bielefeld University, Faculty of Biology / Center for Biotechnology, Algae Biotechnology & Bioenergy Group, Bielefeld, Germany)* Metabolic engineering of microalgae as green cell factories for fuel production

11:50–12:20 **Győző Garab** *(Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary)* Lipid polymorphism in plant thylakoid membranes

12:20–12:45 **Amarendra N. Misra** *(Khallikote (Cluster) University, Berhampur, Odisha, India)* Source sink relationship: can obnoxious gaseous pollutants such as nitric oxide become a source for enhancing photosynthetic productivity in marginal land – a hypothesis

12:45–13:10 **Anjana Jajoo** *(Devi Ahilya Vishwavidyalaya, Indore, India)* Effects of low pH on photosystem I

13:10–14:00 Lunch

Session 3

Chairpersons: Győző Garab (Hungary), R. P. Sharma (India)

14:00–14:30 **Venkata Mohan S.** *(Bioengineering and Environmental Science Lab, EEFF Department, CSIR-Indian Institute of Chemical Technology, India)* Biohydrogen production in the nexus of acidogenesis and photosynthesis: Lab to pilot scale studies

14:30–14:55 **J. S. S. Prakash** *(Department of Biotechnology and Bioinformatics, School of Life Sciences, University of Hyderabad, Hyderabad, India)* Toxin-antitoxin mediated programmed cell death in the cyanobacterium *Synechocystis*

14:55–15:15 **Maria Borisova-Mubarakshina** *(Institute of Basic Biological Problems, RAS, Pushchino, Moscow Region, Russia)* Plant acclimation to environmental light conditions: role of STN7 kinase

15:15–15:35 **Arjun Tiwari** *(Department of Biochemistry, Molecular Plant Biology, University of Turku, Turku, Finland)* Photoinhibition of photosystem I provides protection from excess electron transfer to molecular oxygen and accelerate dissipation of excess absorbed energy

15:35–15:55 **S. D. S. Murthy** *(Department of Biochemistry, Sri Venkateswara University, Tirupati, India)* Toxic effects of mercury on primary processes of photosynthesis in the cyanobacterium *Spirulina platensis*

Group Photo

15:55–16:15 Coffee Break (20 minutes)

Session 4

Chairpersons: Seiji Akimoto (Japan), Arjula R. Reddy (India)

16:15–16:45 **Tatsuya Tomo** *(Graduate School of Science, Tokyo University of Science, Tokyo, Japan)* New chlorophylls in the primary processed of photosynthesis

16:45–17:10 **Seiji Akimoto** *(Graduate School of Science, Kobe University, Kobe; Japan)* Changes in light-harvesting and energy-transfer processes in response to CO_2 concentrations

17:10–17:35 **Yutaka Shibata** *(Graduate School of Science, Tohoku University, Sendai, Japan)* Single-molecule spectroscopy study on photosystem I at low temperatures

17:35–19:00 Poster viewing/discussion

Chairpersons: Barry Bruce (USA), Marian Brestic (Slovakia), Marc Nowaczyk (Germany), Raimund Fromme (USA), Iwane Suzuki (Japan), Seiji Akimoto (Japan), Kostas Stamatakis (Greece), Vasiliy Goltsev (Bulgaria), Cosmin Sicora (Romania), Marek Živčák (Slovakia), Anjana Jajoo (India), Rajagopal Subramanyam (India), Tripathy B. C. (India), Tatsuya Tomo (Japan), Venkata Mohan S. (India)

19:30–22:30 Banquet Dinner

NOVEMBER 2 (THURSDAY – 4TH DAY)

City Tour

NOVEMBER 3 (FRIDAY – 5TH DAY)

Session 1

Chairpersons: Anatoly Tsygankov (Russia), B.C. Tripathy (India), Arjula R. Reddy (India)

9:00–9:30 **Renate Scheibe** *(Faculty of Biology and Chemistry, Department of Plant Physiology, University of Osnabrueck, Osnabrueck, Germany)* Controlling alternatives for photosynthetic electron flow to improve yield

9:30–10:00 **L. C. Rai** (*DAE Raja Ramanna Fellow & DST JC Bose National Fellow (Centre of Advanced Study in Botany, Institute of Science, Banaras Hindu University, Varanasi, India)* AhpC (alr 4404) confers abiotic stress tolerance in cyanobacteria by modulating photosynthesis and antioxidative protein network

10:00–10:25 **Cosmin Sicora** *(Biological Research Center Jibou, Jibou, Salaj County, Romania)* Functional diversity of cyanobacterial D1 proteins

10:25–10:50 **Prabhat Kumar Sharma** *(Department of Botany, Goa University, Goa, India)* Cu nanoparticles and bulk copper have different mechanism to affect growth and photosynthesis in rice plants

10:50–11:10 Coffee Break (20 minutes)

Session 2

Chairpersons: Yutaka Shibata (Japan), Attipalli R. Reddy (India)

11:10–11:25 **Howe-Siang Tan** *(Division of Chemistry and Biological Chemistry, School of Physical and Mathematical Sciences, Nanyang Technological University, Singapore)* Excitation energy transfer processes in plant light harvesting complexes studied by multidimensional electronic spectroscopy

11:25–11:50 **Basanti Biswal** *(Laboratory of Biochemistry and Molecular Biology, School of Life Sciences, Sambalpur University, Jyotivihar, Odisha, India)* Loss in photosynthesis reprograms cellular metabolism to sustain sugar homeostasis in *Arabidopsis thaliana* during senescence and stress response: Induction of cell wall hydrolases

11:50–12:15 **Kentaro Ifuku** *(Graduate School of Biostudies, Kyoto University, Kyoto, Japan)* The PsbP- and PsbQ-family proteins as assembly factors for photosynthetic apparatus

12:15–12:40 **Hitoshi Nakamoto** *(Department of Biochemistry and Molecular Biology, Saitama University, Japan)* Molecular chaperones and stress tolerance in cyanobacteria: role of chaperone paralogs/cognates in the evolution of cyanobacteria

12:40–13:00 **Deepshikha Gupta** *(Department of Plant Science, University of Hyderabad, Hyderabad, India)* Glucose induces photosynthetic damage leading to viable but non-culturable (VBNC) state in *Rubrivivax benzoatilyticus* JA2^T

13:00–14:00 Lunch

Session 3

Chairpersons: Ch. Venkata Ramana (India), Anjana Jajoo (India), Rajagopal Subramanyam (India), JSS Prakash (India)

14:00–14:30 **Anatoly Tsygankov** *(Institute of Basic Biological Problem RAS, Pushchino, Russia)* Hydrogenase electrode based on HydSL hydrogenase from *Thiocasa roseopersicina* with high current density

14:30–14:55 **Misato Teramura** *(Graduate School of Life Sciences, Ritsumeikan University, Kusatsu, Shiga, Japan) In vitro* assay of stereoselective enzymatic reactions in bacteriochlorophyll biosynthetic pathways

14:55–15:20 **Toivo Kallas** *(University of Wisconsin Oshkosh, Oshkosh, WI, USA; Madurai Kamaraj University, Madurai, Tamilnadu, India)* Advances in terpene bioproduction in fast-growing cyanobacteria

15:20–15:35 **Debashree Sengupta** *(Department of Plant Sciences, University of Hyderabad, Hyderabad, India)* A proteomic-based insight into the role of pod wall in regulating carbon allocation and seed filling in soybean under potassium iodide-simulated terminal drought stress

15:35–15:50 **Sunil Bobba** *(Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad, India)* Chloroplast and mitochondrial interactions: possible roles of nitric oxide and reactive oxygen species in mesophyll protoplasts of pea (*Pisum sativum*)

15:50–16:05 **Elsinraju Devadasu** *(Department of Plant Sciences, University of Hyderabad, Hyderabad, Telangana, India)* Unravelling the photosynthesis efficiency and lipid biosynthesis enzymes of *Chlamydomonas reinhardtii* under iron deprivation

16:05–16:20 Coffee Break (15 minutes)

Session 4

Chairpersons: Ch. Venkata Ramana (India), Anjana Jajoo (India), JSS Prakash (India)

16:20–16:32 **Sreeharsha Rachapudi V.** *(Department of Plant Sciences, University of Hyderabad, India)* Enhanced photosynthetic carbon assimilation and antioxidative efficacy favoured sustained growth of drought stressed *Pigeonpea* under elevated CO_2

16:32–16:44 **Aswani Vetcha** *(Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad, India)* Consequences of disturbance in chloroplast or mitochondrial redox in leaf discs of pea, *Pisum sativum*

16:44–16:56 **Panchsheela Nogia** *(Department of Biological Sciences, Birla Institute of Technology and Science, Pilani, India)* Transient expression and localization of a cyanobacterial bicarbonate transporter BicA into chloroplast of *Nicotiana benthamiana*

16:56–17:08 **Srilatha Nama** *(Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad, India)* Long term exposure of high light induced changes in thylakoid organization and their photosynthetic parameters from *Chlamydomonas reinhardtii*

17:08–17:20 **Shabbir Ahmad** *(Department of Plant Science, School Life Science University of Hyderabad Telangana, India)* Characterization of novel L-tryptophan based melanin from *Rubrivivax benzoatilyticus* JA2

17:20–17:35 Coffee Break (15 minutes)

17:35 Special Events

The awards will be presented to young researchers who have done outstanding research in the field of photosynthesis research for sustainability and biohydrogen. All young researchers, including PhD students and Post-Docs, may compete for awards.

Winners will be selected by the committee according to recommendation of chairpersons of poster sections.

Committee (chairpersons of poster sections): Barry Bruce (USA), Marian Brestic (Slovakia), Marc Nowaczyk (Germany), Raimund Fromme (USA), Iwane Suzuki (Japan), Seiji Akimoto (Japan), Kostas Stamatakis (Greece), Vasiliy Goltsev (Bulgaria), Cosmin Sicora (Romania), Marek Živčák (Slovakia), Anjana Jajoo (India), Rajagopal Subramanyam (India), Tripathy B. C. (India), Tatsuya Tomo (Japan), Venkata Mohan S. (India)

Awards for Young Talents (16 awards/prizes) and Closing Ceremony

Committee: Julian J. Eaton-Rye (Secretary of ISPR, New Zealand), Sergey Shabala (Australia), Tatsuya Tomo (Japan), Anatoly Tsygankov (Russia), Johannes Messinger (Sweden), Rajagopal Subramanyam (India), Yuichiro Takahashi (Japan), Govindjee (USA), Suleyman Allakhverdiev (Russia)

19:00 Dinner

Part 1.
Photosynthesis Research for Sustainability

Section 1.1: Primary Processes of Photosynthesis

Lecture

Changes in light-harvesting and energy-transfer processes in response to CO_2 concentrations

Seiji Akimoto[1*], Shiho Ikeda[1], Shimpei Aikawa[2,3], Akihiko Kondo[3]

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Primary processes of photosynthesis alter in response to changes in environmental conditions. Pigment compositions change depending on light quality, light quantity, and, nutrient. In the cyanobacterium *Anacystis nidulans* (*Synechococcus* sp.), the content ratio of phycocyanin to chlorophyll *a* (PC/Chl) is reduced under strong orange light, and increased under strong red light [1]. Nitrogen-deficiency induced degradation of phycobilisome in cyanobacterial cells, resulting in a decrease of PC/Chl [2]. It is also reported that CO_2 concentration affects pigment composition; PC/Chl is higher under 3% CO_2 partial pressure than that under 0.2% [3]. We previously examined changes in primary processes under different light or nutrient conditions by means of time-resoled fluorescence spectroscopy, which is a useful technique to directly examine energy transfer in photosynthetic organisms. In the present study, we will discuss differences in light-harvesting and energy-transfer processes in *Synechocystis* sp. PCC 6803 cells grown under different CO_2 concentrations.

1. A.K. Ghosh, Govindjee, *Biophys. J.* 6 (1966) 611
2. M.M. Allen, A.J. Smith, *Arch. Microbiol.* 69 (1969) 114
3. A. Manodori, A. Melis, *Plant Physiol.* 74 (1984) 67

Lecture

Structure of the symmetric photosystem from *Heliobacterium modesticaldum*

Raimund Fromme

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Reaction centers are protein-pigment complexes which drive photosynthesis by converting light into chemical energy. In the process of evolution the reaction centers have diverged into different classes. The presented structure is the most ancestral and can therefore be called photosystem A [1]. The 2.2-angstrom resolution X-ray structure has two subunits of PshA and two of the single transmembrane helix PshX arranged in the homodimer which exhibits perfect C_2 symmetry.

54 bacteriochlorophyll and 2 carotenoids molecules are capturing the light in the antenna system, the reaction center consists of 6 (bacterio)chlorophyll molecules which perform charge separation and stabilization before the electron transfer takes place to the shared iron-sulfur cluster in the center of symmetry.

This photosystem A structure gives us the first glimpse to evolution of photosynthesis in early earth.

1. Christopher Gisriel, Iosifina Sarrou, Bryan Ferlez, John H. Golbeck, Kevin E. Redding and Raimund Fromme (2017) *Science* 357, pp. 1021-1025

A personal story about *Photosynthesis*

Govindjee

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My interest in photosynthesis research has its beginning in my MSc studies at Allahabad University, India, in early 1954 when our Plant Physiology Professor, Shri Ranjan, asked us to present *a term paper* and *a seminar* on a topic of interest to us – I chose "The R*ole of Chlorophyll (Chl) in Photosynthesis"* – where I discussed its chemistry and function, but what intrigued me most was: Why there was a huge drop in the maximum quantum yield of photosynthesis beyond 680 nm (the "Red Drop"),where Chl *a* was still absorbing light! It is this curiosity that led me to enter the field of photosynthesis, and to work with Robert Emerson for my Ph.D. degree at the University of Illinois at Urbana-Champaign (UIUC), beginning in Sept. 1956. It turned out that Emerson was already solving the mystery of the "Red Drop", when I reached there, and by 1957 he had discovered the "*Emerson Enhancement Effect*", suggesting the existence of two-light reactions and two pigment systems in photosynthesis! Rajni joined Emerson's lab in September 1957, to be his PhD student. Neither of us could finish our PhD under Emerson since on Feb. 4, 1959, he died in a plane crash. Eugene Rabinowitch accepted both of us as his graduate students. Working under Rabinowitch, I proved, in 1960, that Emerson's short-wave system was run by a short wavelength form of Chl *a* : Chl *a* 670, published in *Science*. Further, Rajni proved that the Emerson Enhancement Effect was indeed in Photosynthesis (i.e. in the Hill reaction), not in Respiration, also published in *Science*.

I also plan to summarize selected highlights of my research (& discoveries), with many wonderful students and scientists, which has led to an understanding of the basics of photosynthesis: Excitation energy transfer down to 4 K; primary photochemistry, in both Photosystem (PS) I and II, within a few picoseconds; the very basis of thermoluminescence, and the use of Chl *a* fluorescence in the understanding of both fast (ms to s) and slow (min) changes including "state changes"; and the unique and exciting role of bicarbonate on the electron acceptor side of PS II. Most of our recent publications can be found on my web site at: `http://www.life.illinois.edu/govindjee/recent_papers.html`; and all chronologically arranged at: `http://www.life.illinois.edu/govindjee/pubschron.html`. Now, the future is in making photosynthesis better to deal with the Global Issues facing us all.

I am thankful to Rajagopal Subramanyam, Suleyman Allakhverdiev, James Barber, Tatsuya Tomo and Julian Eaton-Rye for this invitation to be here at the University of Hyderabad. I dedicate this talk to my mentors Robert Emerson (1903–1959) and Eugene I. Rabinowitch (1901–1973).

Lecture

Does the first phase of salt stress affect the osmotic and photosynthetic enzyme systems? A review

Ashwani Kumar, Stephan Jung, Sven Schubert*

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Salt stress is one of the major constraints that limit crop productivity worldwide. In most saline environments, NaCl is the predominant salt species which inhibits cell division and expansion, resulting in slower cell growth and smaller plants. Under saline conditions, plants suffer both osmotic stress and stress caused by disorder of ion homeostasis. Therefore, a salt-resistant plant should possess mechanisms to adapt to osmotic and ion stress. During a long-term breeding program for salt-resistant maize genotypes we established an inbred line of maize with an extremely high capability to restrict Na^+ accumulation in maize leaves [1]. Previous analyses showed a significant increase in PEP-carboxylase activity in young shoots of resistant maize genotypes. Plants of the resistant maize (*Zea mays* L. hybrid SR 03) and wheat (*Triticum aestivum* L. cv. Thasos) were grown under two different light intensities. Analyses of sucrose concentrations showed an increase in the saline treatment of both genotypes independent of the light intensity. Results of sucrose concentrations led to the deduction that an increase in PEP-carboxylase activity was not required for sugar metabolism. Independent of light intensity, alkalinity and malate concentrations were decreased only in wheat. It was concluded that an enhancement of PEP-carboxylase activity in young shoots of maize supports organic acid metabolism under salt stress [2]. Recent studies suggest that under salt stress PEP carboxylase activity was significantly increased in sink leaves and shoot apex of maize, whereas no significant effect was observed in the root apex. In conclusion, PEP carboxylase may have an anaplerotic function supporting the demand for metabolites in sink shoot tissues of young maize plants under salt stress [3]. A review of our work on these aspects shall be presented.

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3. Hütsch, B. W., T. Osthushenrich, F. Faust, A. Kumar, and S. Schubert (2016) *J. Agron. Crop Sci.* 202: 384-393.

Lecture

New chlorophylls in the primary processed of photosynthesis

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There are a number of diversity in photosynthetic algae. Diversity also exists in the pigment species in photosystems. *Acaryochloris* marina and *Halomicronema hongdechloris* are unique cyanobacteria that differ from the majority of photosynthetic organisms by having chlorophyll (Chl) *d* and Chl *f*, respectively. Chls play essential roles in light harvesting, energy transfer, charge separation and electron transfer in Photosystem (PS) I and II. Chl *d* and *f* has the structure [3-formyl]-Chl *a* and [2-formyl]-Chl *a,* respectively. Chl *d* and *f* absorb light with a wavelength up to 30 and 40 nm red-shifted from Chl *a*, respectively.

Despite of great interest in these unique Chls, many questions related to function of such pigments in primary photosynthetic processes are still not elucidated.

In the case of Chl *d* type cyanobacterium, a small number of Chl *a* were always bound in PS I and II complexes. The energy of Chl *d* is lower than of Chl *a*. If the Chl *a* is involved in the charge separation process, our current understanding of the overall energetics of the PS II would need to be modified.

In the case of Chl *f* type cyanobacterium, the Chl content varied under different light conditions. When under far-red light (>700 nm), the Chl *f* content increased to 10%–12.5% of the total Chl. When under white fluorescent light, the Chl *f* content decreased negligibly. To understand the accumulation process of Chl *f*, we can understand the universal energy transfer process in photosynthetic reaction.

In this meeting, we will discuss the latest advances in the field of Chl *d* and Chl *f* research and their role in primary photosynthetic process.

This study was supported by the Grants-in-Aids for Scientific Research from JSPS Nos: 26220801, 17K07453 (to TT), and by the Russian Science Foundation No: 14-14-00039 (to SIA).

Poster

Light-induced conformational changes and rate-limitation in photosystem II reaction centers. The origin of variable chlorophyll fluorescence

Melinda Magyar[1*], Gábor Sipka[1], Alberto Mezzetti[2], László Kovács[1], Bettina Ughy[1], Qingjun Zhu[3], Guangye Han[3], Vladimír Špunda[4], Petar H. Lambrev[1], Winfried Leibl[2], Jian-Ren Shen[3, 5], Győző Garab[1, 4]

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Whereas the structure and function of Photosystem II (PSII) and its reaction center (RC) is well known [1, 2], some important questions remain to be elucidated, e.g.: (i) does PSII RC possess structural dynamics associated with its function, and (ii) does the variable chlorophyll fluorescence reflect solely the reduction of the first quinone acceptor (Q_A) [3]?

Here we report on chlorophyll fluorescence transients elicited by single-turnover saturating flashes (STSFs) in DCMU-treated samples between 170 and 300 K. We found that a STSF raised the fluorescence yield from F_o to F_1 and further flashes were required to reach the maximum level F_m. The fluorescence increment $(F_m F_1)/(F_m - F_o)$ increased with decreasing temperatures. F_1 relaxation due to charge recombination involving Q_A^- was observed only above 230 K; in contrast, the increment relaxed at all temperatures. In experiments using double flashes with waiting time t_w between them, in a train of STSFs, a striking finding was that the rise of the fluorescence yield from F_1 to F_m depended on t_w revealing a previously unknown, temperature-dependent rate limitation in PSII.

We conclude that the reduction of Q_A is a necessary but not sufficient condition to generate F_m and light-induced events, probably conformational changes [4], detected by fast-scan FTIR, occur in the F_1-to-F_m rate-limited transition. We hypothesize that dielectric relaxation in the RC matrix exposed to the strong local electric field due to charge separation explains the observed phenomena [5], in which STSF-induced ultrafast transient electric field effects and/or dissipation-induced thermal transients may also play a role.

1 Cardona, T. et al. (2012) BBA 1817:26
2 Suga, M. et al. (2015) Nature 517:99
3 Govindjee & Stirbet (2012) Photosynth Res 113:15
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5 Malferrari, M. et al. (2013) BBA. 1827:328

Section 1.2: Structure, Function and Biogenesis of the Photosynthetic Apparatus

Lecture

The journey through the structure-function of the complex "cytochrome b_6f": Personal perspective dedicated to Prof. William A. Cramer

Rachna Agarwal

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The energy transducing cytochrome b_6f complex of oxygenic photosynthesis is a structural and functional dimer, performing electron transport as well as proton translocation across the thylakoid membranes. Dr. William A. Cramer's laboratory has been actively addressing the finer nuances of the structure-function of this complex in parts as well as a whole. From optimizing the purification conditions for the maximal yield of an active complex from spinach and cyanobacteria, to solving the crystal structure from *Mastigocladus* and *Nostoc*, their road to discovery has spanned over 3 decades. The difficulties encountered while crystallization attempts on the cyanobacterial cytochrome b_6f complex were tackled effectively by "putting the lipids back" and its first crystal structure was published from his lab in 2003. The crystal structure of this complex showed a central core of 4 large subunits (Cyt b_6, Cyt *f*, Iron Sulphur Protein and Subunit IV) surrounded by 4 small subunits, per monomer. Additionally, 7 prosthetic groups per monomer (including one molecule each of chlorophyll *a* and β-carotene) were also observed. The ISP, through its large soluble domain, showed a unique domain swapping across the two monomers which was postulated to hold them together as its cleavage would invariably lead to monomerization and loss of electron transport activity. However, our studies with cytochrome b_6f from *Fremyella diplosiphon* showed that intact ISP serves primarily a functional role rather than holding the two monomers together in this system. Based on the known crystal structures from other sources, intermonomer hydrophobic interactions mediated by the interfacial polypeptides as well as lipids were suggested to contribute to dimer stability in cytochrome b_6f.

Lecture

Enhanced superoxide production in cytochrome b_6f complex of oxygenic photosynthesis and its role in plant physiology

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Reactive oxygen species (ROS) produced in chloroplasts have a versatile role implicated in regulation of photosynthetic electron transport and in retrograde signaling from chloroplasts to the nucleus. The major source of superoxide ($O_2^{\bullet-}$) generated in chloroplasts is photosystem I. However, other sources may contribute to the overall ROS production or have specific functions. Our study characterized $O_2^{\bullet-}$ production in the cytochrome b_6f complex. The specific rate of $O_2^{\bullet-}$ production in purified cytochrome b_6f complex, normalized to the rate of electron transport, has been found to be more than an order of magnitude greater than that measured in isolated yeast respiratory bc_1 complex. The $O_2^{\bullet-}$ production in the *bc* complexes has been proposed to be manifested as a bypass reaction of the bifurcated two electron oxidation reaction implicated in Q cycle electron transfer [1].

Despite the fact that general principles of the Q-cycle reactions are considered to be well established, the specific sequence of oxidation-reduction events leading to $O_2^{\bullet-}$ production remains vague. The larger rate of superoxide production in the b_6f complex could be a consequence of a more prolonged plastoquinone/plastoquinol residence time in the binding niche near the Rieske protein iron-sulfur cluster, resulting from occlusion of the quinone portal by the phytyl chain of the unique bound chlorophyll or an altered environment of the proton-accepting glutamate believed to be a proton acceptor from semiquinone [2].

The cytochrome b_6f complex appears as an important source of ROS in the thylakoid lumen. Altered levels of ROS production are alleged to convey redox signals from the lumen to the stromal side of the thylakoid membrane in the regulation of photosynthetic state transitions, and conceivably might be involved in signalling from the organelle to the cytosol and nucleus.

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Lecture

Ironies in photosynthetic electron transport: the Cytochrome b_6f lipoprotein complex

William A. Cramer[1], S. D. Zakharov[1], S. I. Singh[1], S. Bhaduri[1], S. Saif Hasan[1], R. Agarwal[1,2], J. P. Whitelegge[1,3]

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Cytochrome *f*, the 'ears' of the b_6f complex, whose atomic structure (1) provided the first insight into that of the entire complex, also provided an initial enigma, in that its structure turns out to be unrelated to that of the mitochondrial cytochrome c_1.The subsequent delay of 8 years in obtaining the solution of the b_6f complex, at least in cyanobacteria (2), which was accompanied by the structure from *C. reinhardtii* (3), was a consequence of repeated over-purification, and consequent de-lipidation. The ubiquitous role of lipid in the structure of hetero-oligomeric integral membrane proteins was pointed out initially for trans-membrane K^+ channels (4), and, shortly after, the requirement of lipid addition for successful crystallization of b_6f complex (5). At 2.5 Å resolution, the distribution of the 23 lipids/lipid binding sites in the b_6f complex could be mapped (6), showing a special role of the anionic sulfo-lipid, a number of lipid binding sites between the protein core conserved in evolution, and the unconserved peripheral hydrophobic picket fence in each monomer, the latter feature a unique feature in a membrane protein. The uneven lipid distribution may explain the heterogeneity of dielectric constants between the 4 hemes in the b_6f (7) and bacterial bc_1 (8) complex.

Activation of an LHCP kinase to regulate the distribution of light energy between the photosystems has been proposed to be a TM signaling system in which the b_6f complex has been proposed to participate through redox interaction with b_6f on the p-side of the membrane (9-12). Some questions about the TM property: although the Stt7 (Ser-Thr) kinase contains a putative 25 residue hydrophobic domain, its TM nature is made questionable by the 4 proline residues in this segment. Similarly, although far-UV circular dichroism studies implied an interaction between b_6f and Stt7 (12), the finding that the kinase is a 332 kDa tetramer (12) make it difficult to envisage its interaction with the p-side of the b_6f complex and a TM topology.

The structure description of the tortuous p-side entry pathway for plastoquinol (13) make it difficult to understand how two quinols can enter and exit the complex in the rate-limiting msec time frame in the absence of a PSII reaction center–cyt b_6f super-complex.

The question of whether the structure of membrane proteins is influenced by the electrical field of approximately 3×10^5 Volts/cm that typically spans biological membranes has been studied using the cyt b_6f complex incorporated into liposomes and planar bilayer membranes.

Studies supported by US NIHGMS-038323.

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Lecture

Lipid polymorphism in plant thylakoid membranes

Győző Garab[1,2*], Bettina Ughy[1], Pieter de Waard[3], Parveen Akhtar[1], Uroš Javornik[4], Christos Kotakis[1], Primož Šket[4], Václav Karlický[2], Zuzana Materová[2], Vladimír Špunda[2], Janez Plavec[4], Herbert van Amerongen[3], László Vígh[1], Henk van As[3], Petar H. Lambrev[1]

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In chloroplast thylakoid membranes, the functional state of which is a bilayer, the non-bilayer lipid monogalatosyl-diacylglycerol (MGDG) accounts for about half of the lipid content. MGDG is known to play key role in the operation of violaxanthin de-epoxidase and is present in most pigment-protein complexes, but this does not explain its high abundance in all oxygenic photosynthetic organisms. It has been hypothesized that non-bilayer lipids, via their segregation capability, regulate the protein-to-lipid ratio of the membranes, and contribute to the structural flexibility of thylakoid membranes (Garab et al. 2000 TIPS). Earlier, using ^{31}P-NMR and steady state and time-resolved fluorescence measurements, using merocyanine 540 (MC540) on spinach thylakoid membranes we have shown the coexistence of a non-bilayer lipid phase and the bilayer phase of thylakoid membranes (Krumova et al. 2008 BBA Biomembranes); and experiments on *dgd1* mutant of Arabidopsis revealed the dependence of membrane stability on the concentration of MGDG (Krumova et al, 2010 Photosynth Res). Here, in a series of experiments, using circular dichroism spectroscopy and time-resolved fluorescence on MC540-stained thylakoid membranes and by applying co-solute treatments of thylakoid membranes, we have further substantiated these conclusions. Our recent ^{31}P-NMR experiments revealed the presence of two isotropic phases and one inverted hexagonal phase, in addition to the bilayer phase and showed that the phase behavior of isolated thylakoid membranes (i) is sensitive to the osmolarity and ionic strength of the medium, (ii) can be modulated by rigidifying the membranes, and (iii) exhibit a marked increase of one of the isotropic phases upon lowering the pH of the medium. We propose a membrane model in which fusion channels at the granum-stroma junctions and lipocalin:lipid assemblies play important roles and in which non-bilayer lipid phases, by adding a new dimension to the structural flexibility of thylakoid membranes, determine the dynamic features of membranes (Garab et al. 2016 in: Lipids in Plant and Algae Development).

Lecture

Targeted mutation of D1 and D2 amino acids residues associated with bicarbonate binding and the protonation of plastoquinone B

Julian J. Eaton-Rye*, Jack A. Forsman, Harvinder Singh, Victor Zhong

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The bicarbonate co-factor of the quinone-Fe-acceptor complex of Photosystem II (PSII) is a bidendate ligand to the non-heme iron. It has been hypothesized that the protonation of the secondary plastoquinone electron acceptor, $Q_{B,}$ proceeds first through D1-His252 and D1-Ser264 to give $Q_B^-(H^+)$ followed by the second proton (resulting in Q_BH_2) being delivered via the D1-His272 and D1-His215 ligands of the non-heme iron. The pathway for the second proton is hypothesized to include two waters: W675A and W1138A in PDB 3ARC. W1138A has hydrogen bonds to both bicarbonate and W675A, and protons are suggested to pass via this route to D1-His272 and D1-His215: furthermore, our targeted mutations in the D1 protein support this interpretation. The W675A water is also hydrogen-bonded to D2-Thr243: additionally, D2-Glu242 interacts with D1-Glu244 that is also hydrogen-bonded to W675A and D2-Lys264 is hydrogen-bonded to D2-Glu242, potentially stabilizing the hydrogen-bond network around W675A. We have introduced mutations at D2-Glu242, D2-Thr243 and D2-Lys264: in these mutants, which assemble near wild-type levels of PSII, Q_A to Q_B electron transfer is substantially slowed while oxygen evolution is depressed by more than 50% but rescued by addition of bicarbonate. Moreover, D2-Tyr244 is hydrogen-bonded to bicarbonate and targeting this residue also disrupts Q_A to Q_B electron transfer. Our results indicate: (1) D2 plays an equal role to D1 in supporting the putative pathway of protons from the cytosol to bicarbonate; and (2) support the hypothesis that bicarbonate is involved in protonation of $Q_B^{2-}(H^+)$ to Q_BH_2 before Q_BH_2 is released to the plastoquinone/plastoquinol pool.

Lecture

The PsbP- and PsbQ-family proteins as assembly factors for photosynthetic apparatus

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The photosynthetic oxygen-evolving reaction catalyzed by photosystem II (PSII) is the basis for the light-to-chemical energy conversion on earth. The oxygen-evolving complex (OEC) proteins are membrane-extrinsic subunits of PSII and optimizes the oxygen evolution. It is known that the composition of OEC proteins is largely differed among photosynthetic organisms. The PsbP and PsbQ proteins are OEC proteins specifically found green plants, including higher plants and green algae. These proteins are thought to have evolved from their cyanobacterial homologs; CyanoP and CyanoQ, respectively. In addition, multiple isoforms and homologs for PsbP and PsbQ proteins have been found in the chloroplast thylakoid lumen. A number of reports from our group and others have suggested that OEC-family proteins have various roles in photosynthetic electron transfer. In particular, some of them functions as assembly factors for photosynthetic apparatus, not only for PSII, but also for PSI and the plastid NADH dehydorogenase-like (NDH) complex operating cyclic electron transport around PSI. These facts suggest that the acquisition of PsbP and PsbQ in PSII involved gene duplication and intensive functional diversification as assembly factors for photosynthetic apparatus. In fact, PsbP seems to have a dual function as an assembly factor and a constitutive subunit for PSII.

Lecture

High-resolution structures of plant and cyanobacterial Photosystem I

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Plant Photosystem I (PSI) is one of the most intricate membrane complexes in Nature. It is comprised of two complexes, a reaction center and light-harvesting LHCI. An atomic-level structural model of higher plant PSI at 2.2 Å resolution has been constructed based on new crystal form. The crystal belongs to $P2_12_12_1$ symmetry space group, with one protein complex in each asymmetric unit. The structure includes 16 subunits and more than 200 prosthetic groups, the majority of which are light harvesting pigments. The model reveals detailed interactions, providing mechanisms for excitation energy transfer and its modulation in one of Nature's most efficient photochemical machine.

Recently we solved the structure of trimeric PSI from *Synechocystis* at 2.5 Å resolution. Several differences between the mesophilic and thermophilic PSI were revealed and the position of lipids between the monomers was determined. Similarly the structure of monomeric PSI was determined. An operon encoding PSI was identified in cyanobacterial marine viruses. We generated a PSI that mimics the salient features of the viral complex containing PsaJ-F fusion subunit. The mutant is promiscuous for its electron donors and can accept electrons from respiratory cytochromes. We solved the structure of the PsaJ-F fusion mutant as well as a monomeric PSI at 2.8 Å resolution, with subunit composition similar to the viral PSI. The novel structures provided for the first time a detailed description of the reaction center and antenna system from mesophilic cyanobacteria, including red chlorophylls and cofactors of the electron transport chain. Our finding extends the understanding of PSI structure, function and evolution and suggests a unique function for the viral PSI.

Lecture

Analysis of Photosystem II Electron Transfer by Redox Polymer/Protein Biophotoelectrochemistry

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Photosystem 2 (PS2) and Photosystem 1 (PS1) are the key components of natural photosynthesis. Light induced charge separation within the photosystems drives electron transfer (ET) from water to NADPH, which is limited by diffusion-dependent electron mediators like quinones and small soluble redox proteins. However, assembly of isolated photosystems and redox-active polymers *in vitro* facilitates diffusion-free ET without additional external bias [1, 2] that enables further engineering of ET pathways. Moreover, protein biophotoelectrochemistry can be used as an analytical tool to study PS2 photoinhibition, e.g. by simultaneous measurement of ET and formation of reactive oxygen species [3] or by direct determination of maximum ET capacity, degradation rate and light-conversion efficiency of different PS2 variants [4].

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2. Hartmann V, Kothe T, Pöller S, El-Mohsnawy E, Nowaczyk MM, Plumeré N, Schuhmann W, Rögner M (2014) Redox hydrogels with adjusted redox potential for improved efficiency in Z-scheme inspired biophotovoltaic cells. *Phys. Chem. Chem. Phys.* 16, 11936–11941
3. Vöpel T, Saw EN, Hartmann V, Williams R, Müller F, Schuhmann W, Plumeré N, Nowaczyk MM, Ebbinghaus S, Rögner M (2016) Simultaneous measurements of photocurrents and H_2O_2 evolution from solvent exposed photosystem 2 complexes. *Biointerphases* 11, 019001
4. Hartmann V, Ruff A, Schuhmann W, Rögner M, Nowaczyk MM; Analysis of Photosystem II Electron Transfer with Natural PsbA-Variants by Redox Polymer/Protein Biophotoelectrochemistry; *submitted*

Lecture

Identification and characterization of a photosystem I assembly apparatus

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Photosystem I (PSI) drives light-induced electron transfer from plastocyanin to ferredoxin and forms a large multi-protein complex. PSI complex of the green alga, *Chlamydomonas reinhardtii*, contains 14 subunits and nine distinct light-harvesting complexes (LHCIs) to form a PSI-LHCI supercomplex. The structure and function of PSI complex have been investigated in detail, but the molecular mechanism by which PSI complex is synthesized remains largely unknown. Chloroplast encoded Ycf3 and Ycf4 are auxiliary factors required for PSI assembly. Ycf3 is a TPR protein and is essential for PSI accumulation in tobacco and *C. reinhardtii*, whereas Ycf4 with two putative transmembrane helices forms a large oligomeric structure and is an important factor for PSI assembly in cyanobacteria and tobacco but essential in *C. reinhardtii*. It is, however, unclear how these factors assist PSI assembly process. Here we generated chloroplast mutants in which HA tag has been fused at C-terminus of Ycf3 (Ycf3-HA) and at N-terminus of Ycf4 (HA-Ycf4) and purified these tagged proteins by affinity spin column in order to identify interacting proteins with Ycf3 or Ycf4. Y3IP1 (Ycf3 interacting protein 1) was pulled-down with Ycf3-HA and small amount of Ycf4. In addition, small amount of PSI RC proteins (PsaA and PsaB), which were newly synthesized, were pulled down. Next, we purified HA-Ycf4 and confirmed that HA-Ycf4 forms a large oligomeric complex (Ycf4 subcomplex) and associates small amount of Ycf3 and Y3IP1. Of interest is that most PSI proteins and LHCI proteins were pulled down. Based on the results obtained above, it is concluded that an initial assembly event is assisted by Ycf3-Y3IP1 subcomplex and the subsequent assembly events to integrate other peripheral PSI proteins and LHCI proteins are facilitated by Ycf4 subcomplex.

Poster

Effect of HA tagged FNR expression in the chloroplast on PSI function and photosynthetic growth in the green alga *Chlamydomonas reinhardtii*

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Photosystem I (PSI) drives light-induced electron transfer from plastocyanin or cytochrome B6f to ferredoxin (Fd), and subsequently reduced ferredoxin transfers its electron to $NADP^+$ oxidoreductase (FNR). FNR thus catalyzes electron transfer between the one-electron carrier Fd and the two-electron carrier $NADP^+$ at the end of the photosynthetic linear electron transport chain. Under strong or fluctuating light conditions, the reducing side of PSI could be over-reduced, which leads to photoinhibition of PSI. It is thus expected that increase of FNR level could enhance photosynthetic performance and/or protect PSI from photodamage. In the present study, we aimed to express additional FNR fused with HA tag in the chloroplast of the green alga *C. reinhardtii* by chloroplast transformation using Fud7, the chloroplast psbA gene deletion mutant, as a host strain. A coding region of FNR without the transit peptide and HA tag at its C-terminus was synthesized using codon usage optimized for the chloroplast genes and the coding sequence was fused with 5' region of psaA gene and 3' region of rbcL gene. The resulting chimeric gene was inserted at a BamHI site of the chloroplast DNA containing the psbA gene and its downstream sequence to construct FNR-HA transforming vector. After biolistic bombardment of the vector, transformants (oxFNR) were selected on photosynthetic condition (on high salt minimum solid medium in the light). The expression of FNR-HA was confirmed by immunoblot using anti-FNR and anti-HA antibodies. The accumulation of FNR and FNR-HA was estimated in cells grown under different light conditions (50, 200 and 500 µmol photons/m^2/sec). It was found that more FNR and FNR-HA were accumulated under stronger light conditions. We also measured P700 photooxidation and re-reduction kinetics and photosynthetic growth of oxFNR cells of oxFNR cells grown under different light conditions.

Poster

Two types of pigment-protein complex cycle during non-leaf green tissues development of mungbean (*Vigna radiata*)

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Photosynthetic pigment-protein complexes are a vital component of light-harvesting machinery of all plants, it including two light-harvesting complexes and two photosystems. In this study, we found non-leaves green tissues (NLGTs) such as cotyledons, testa and pods, have different expression pattern of pigment-protein complexes and incomplete photosynthetic ability. The deficiency of pigment-protein complexes will recover in cotyledons after seed germination and light irradiation, indicating the pigment–protein complexes were fully recovered and formed a new cycle, we temporary called the cycle is "complete pigment-protein complex cycle (CPPCC)"; on the other hand, testa and pods generate similar green tissues that have incomplete PSI and PSII pigment–protein complexes, but it can't recover after germination and light irradiation, we temporary called the cycle is "semi-complete pigment-protein complex cycle (SPPCC)".

Many physiological parameters and photosynthetic pigments were maintained in higher level during NLGTs development (stage I-III) and then down regulated very fast (stage IV-V) until the signals were undectable. After seeds germination in light condition, like CPPCC, the parameters and photosynthetic pigments were recover. In contrast, in SPPCC pathway, the parameters and photopigments cannot recover in NLGTs.

Poster

Characterization of Alb3.1 mutant, BF4, defective in the LHC complexes in the green alga, *Chlamydomonas reinhardtii*

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The unicellular green alga, *Chlamydomonas reinhardtii*, contains several peripheral antenna complexes associating both chlorophylls *a* and *b*; the major antenna complexes for photosystem II (PSII), LHCIIs (Type I, II, III, and IV) and minor antenna complexes, CP26 and CP29 as well as nine antenna complexes for PSI, LHCIs (LHCA1-9). A low fluorescent mutant, BF4, which is pale green, shows higher Chl *a*/*b* ratio and grows photosynthetically. Immunodetection using specific antibodies against all antenna proteins revealed that the four major LHCIIs were reduced to 25–50%, CP26 and CP29 were reduced to <25% on a Chl basis. No state transition was observed in this mutant. We also found that the nine LHCIs are significantly reduced to <25% except for LHCA5 and LHCA6 that accumulated at around 25% of wild-type level on a PSI basis. These observations suggest that the accumulation of LHC complexes was significantly impaired in BF4. Complementation test indicated that BF4 contains a mutation in *Alb3.1* gene. ALB3.1 protein mediates an important role in insertion, folding and assembly of LHC proteins. To elucidate the molecular mechanism by which ALB3.1 assists LHC assembly, we generated complementation mutants in which ALB3.1 protein fused with 1xHA or 3xHA tag is expressed using paromomycin resistance gene as a selectable marker. The complemented mutants recovered the accumulation of the major and minor antenna proteins as well as LHCI proteins, suggesting that HA tagged ALB3.1 is functional. Subsequently thylakoid membranes from the complemented cells were isolated and solubilized. HA-ALB3.1 was purified from the thylakoid extracts by affinity spin columns and the resulting preparation was analyzed by SDS-PAGE and immunoblotting. We are currently optimizing the solubilization and purification conditions to detect interacting proteins with ALB3.1.

Poster

Xanthophylls deficiency affects the microdomain organization of thylakoid membrane and state transitions in the cyanobacterium *Synechocystis sp.* PCC 6803

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Although several effects of xanthophylls on the physico-chemical properties of the thylakoid membrane have been observed, their influence on the pigement-protein interactions of the photosynthetic complexes has not been clarified yet. Recently, we have revealed that xanthophylls can stabilize the photosystem oligomers, although no xanthophylls have been observed in these proteins. This effect could be explained by the direct interaction of peripheral xanthophylls with a specific part of the transmembrane protein subunits or by influencing the lipid environment of the complex. In this study, we further investigate the relationship between xanthophylls and supramolecular organization *in situ* in the thylakoid membrane of cyanobacterium *Synechocystis* sp. PCC 6803 by confocal microscopy

An altered distribution of pigment-protein complexes in thylakoid was observed in the xanthophyll deficient mutant cells. Two kinds of pigment-protein domains were distinguishable based on chlorophyll and phycobilin autofluorescence shown in their simultaneous high or low emissions. In the xanthophyll deficient mutant, this kind of microdomains organization was highly affected and thylakoid membrane structure was changed. Moreover, we have recorded a total increase in phycobilisomes emission of cells that was explained as a result of loosely bound phycobilisomes to photosystems. Finally, the modified thylakoids caused by lacking of xanthophyll affected mechanism of state transitions, as it is deduced from fluorescence inductions. Our results show that xanthophylls are important for the proper organization of pigment-proteins in thylakoids and affect the mechanism of state transitions regulating excitation flow between photosystems.

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2. Tunde N. Toth et al. (2015) BBA-Bioenergetics. 1847:1153-1165.

Poster

Xanthophyll cycle is the adaptive strategy in the leaves of variegated Ficus

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The white sector in the variegated leaves are studied by the variegated mutant lines, but little attention are paid in the leaves of natural plant. In present study, we analyzed the biosynthetic capacity of chlorophyll and the intermediates of xanthophyll cycle in the green and white sectors of variegated leaves of milky stripe fig (*Ficus microcarpa* cv. milky stripe). Results showed that in the white sector the ratio of carotenoid to chlorophyll was 5.3-fold higher than that in the green sector. The rate of degradation of protoporphyrin IX (PPIX), magnesium protoporphyrin IX (MGPP), and protochlorophyllide (Pchlide) in the white sector was higher than that in the green sector. δ-Aminolevulinic acid (ALA)-supplementation test indicates that the chlorophyll biosynthesis between ALA and Pchlide was partially impaired in the white sector, but is remarkably impaired in the steps after Pchlide. This study revealed the deficient process of photosynthesis and the importance of xanthophyll cycle in the white sector of natural leaves.

Section 1.3: Bacterial Photosynthesis and its metabolism

Lecture

Understanding in situ light-harvesting strategies

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In modern world, there is a pressing need for sustainable energy technologies with minimal stress on the environment. This motivates to look for the solutions used by the Nature itself. Photosynthesis is a universal process of conversion light energy into chemical form, providing all food, feed as well as oxygen we breathe. Photosynthesis begins with harvesting solar energy by excitons – collective excitations of special pigment arrangements in light-harvesting pigment-protein complexes. Here we discuss the permanent changes observed in the spectra of cyclic LH2 antenna complexes from purple photosynthetic bacteria that occur upon high-fluency optical irradiation in the presence of oxygen. The complexes were studied applying a range of steady-state and picosecond time-resolved optical spectroscopies. Experimental data were complemented with exciton model simulations. The most important spectral effects observed – bleaching and blue shifting of the B850 exciton absorption and fluorescence bands, and the coordinated emergence of a new exciton band around 700 nm – were associated with the photooxidation of various numbers of bacteriochlorophyll *a* molecules mostly in the B850 domain of LH2 containing 16 or 18 closely coupled chromophores in different species. Our analyses as well as the literature biochemical evidence both imply that these changes occur without noticeable damage to the surrounding protein scaffold. A prospective non-invasive method for an *in situ* optical control of the properties of photosynthetic excitons was thus demonstrated. It remains to be seen whether the discovered photo-induced spectral modulation principle also has a utilization potential for present or future photonics technology.

Lecture

Insights into the catalysis of ferredoxin-NAD(P)$^+$ oxidoreductases from *Rhodopseudomonas palstris* with NADP$^+$/H based on kinetic and structural analyses

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Rhodopseudomonas palstris is known as a versatile bacterium performing non-oxygenic photosynthesis with organic or sulfur compounds as an electron source, or non-photosynthetic autotrophic or heterotrophic growth with hydrogen or organic compounds, respectively, as an energy source. The bacterium is also capable of nitrogen assimilation and degradation of several aromatic compounds. These versatile metabolic processes often use a small iron-sulfur protein ferredoxin as an electron mediator. In the *R. palstris* genome, six putative ferredoxin genes and three putative ferredoxin-NAD(P)$^+$ oxidoreductase (FNR) genes as well as pyruvate ferredoxin oxidoreductase gene are found. The RPA3954 gene encodes a homo-dimer type FNR (*Rp*FNR) with a significant structural homology to the bacterial NADPH-thioredoxin reductases found in green sulfur bacteria and firmicutes. In this presentation, we report crystal structure analysis of *Rp*FNR and pre-steady state kinetic studies of NADP$^+$/NADPH reduction/oxidation reactions with implication of NADP$^+$/H complex formation.

Mixing oxidized *Rp*FNR with NADPH yielded a formation of CT complexes followed by a reduction. *Rp*FNR was reduced partially at equilibrium in the presence of excess NADPH. Mixing reduced *Rp*FNR with NADP$^+$ resulted in a rapid formation of CT complexes followed by a reduction of NADP$^+$. The kinetic analyses indicated that the rate-limiting steps were the hydride-transfer process in both directions. The hydride transfer rate for NADPH-oxidation direction was comparable to that of NADP$^+$ reduction direction, indicating the reaction between *Rp*FNR and NADP$^+$/NADPH was reversible, which differs from FNRs from *Bacillus subtili*s and *Chlorobaculum tepidum* [1, 2]. Crystal structure analysis revealed that *Rp*FNR share the NADPH binding mode with *B. subtilis*, but C-terminal region differed from the other FNRs. The structure-function relation of *Rp*FNR regard to the CT complex formation and spectral features will be discussed.

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2. Seo et al. (2016) BiochimBiophysActa. 1857(6), pp. 678-687

Lecture

Characterization of novel L-tryptophan based melanin from *Rubrivivax benzoatilyticus* JA2

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Melanin is an enigmatic pigment found mainly in animals, fungi and some bacteria and it is a complex polymer that protects against UV radiations and other physiological stresses. Bacterial melanins are rarer and derived mainly from L-tyrosine or L-phenylalanine and play an important role in the protection of organism under different physiological condition. We have discovered a novel melanin from a phototrophic bacterium, *Rubrivivax benzoatilyticus* JA2 when fed with L- tryptophan as the sole source of nitrogen under aerobic conditions. The draft genome sequence of *Rubrivivax benzoatilyticus* JA2 did not show tyrosinase only few oxidoreductase gene observed. The novel melanin is brown colored, amorphous, insoluble in water or organic solvents and it is soluble in 1N NaOH /KOH or partially soluble in dimethyl sulfoxide (DMSO). Melanin produced from L-tryptophan was characterized based on the polychemical analysis (UV, IR, NMR) and alkaline hydrolysis of melanin. The melanin thus produced mainly consists of indole and indole derived products. A series of inhibitor were used (namely sodium azide, kojic acid, quercetin, glyphosate, and Sulcotrion) confirm the melanin biosynthetic route. The inhibitor studies confirmed that melanin produced in preence L-tryptophan is not synthesised from canonical melanin biosynthetic route in strain JA2 and possibly.synthesied via alternative route. To the best of our knowledge, this is the first report of melanin production from L-tryptophan in bacteria.

Lecture

In vitro assay of stereoselective enzymatic reactions in bacteriochlorophyll biosynthetic pathways

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In nature, chlorophylls (Chls) allow phototrophs to drive photosynthesis. A variety of Chl pigments found in the organisms are synthesized by stepwise enzymatic reactions, and the resulting π-conjugation degree and peripheral substituents of pigments affect their optical properties. BChl *a* is one of the most widespread photosynthetically active pigments, which is found in reaction centers and light-harvesting antennas of many photosynthetic bacteria. The chemical structure of BChl *a* is characterized by the reduced ring B and an acetyl group at the C3 position. This 3-acetyl group is introduced by the hydration of the 3-vinyl group by BchF homologous enzymes followed by oxidation of the resulting 1-hydroxyethyl group by a BchC enzyme ($3\text{-}CH{=}CH_2 \rightarrow 3\text{-}C^*H(OH)CH_3 \rightarrow 3\text{-}COCH_3$) [1]. On the other hand, BChls *c*/*d*/*e*/*f* are known as Chl derivatives possessing a 1-hydroxyethyl group at the C3 position which are found in unique light-harvesting antenna complexes, called chlorosomes, utilized in green sulfur bacteria, filamentous anoxygenic phototrophs, and acidobacteria cells. The hydration process by BchF-homologs synthesizes a chiral carbon at the $C3^1$ position [2, 3] However, the effect of the stereochemistry at the $C3^1$ positon *in vivo* still remains poorly understood. To clarify these processes, we studied the *in vitro* activities of BchF homologous hydratases and BchC enzymes.

Recombinant BchF homologous hydratases, BchF and BchV, and BchC oxidoreductase derived from a green sulfur bacterium *Chlorobaculum tepidum* were used for *in vitro* assays. We found that BchF/V and BchC catalyzed stereoselective hydration and oxidation/reduction, respectively. Based on the biochemical characterizations, we will discuss how these enzymes play a role in the biosynthetic pathway of BChls and the effect of the stereochemistry at the $C3^1$ position *in vivo*.

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Poster

Genome sequence of *Rhodobacter johrii* JA192

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Bacterial endospores are believed to be produces only by the members of the phylum Firmicutes. However, some of the recent discoveries indicated that the phenomenon is not confined to the members of the phylum. Firmicutes and some of the members of the other phyla also are capable of producing endospore like structures. One such Gram-stain-negative alphaproteobacterium, *Rhodobacter johrii* JA192 produces endospore like structures, however the molecular insights of sporogenesis is still not known. We are investigating the molecular insights of endospore formation starting from its draft genome sequence through annotation and *In silico* analysis. Genome was sequenced and annotations were performed with *de novo* assembled sequence using the RAST (Rapid Annotation using Subsystem Technology) servers and through the NCBI Prokaryotic Genome Automatic Annotation Pipeline (PGAAP). The scaffolds sequences of the draft assembly have been deposited at GenBank (Submission number "SUB1585468"; Bio Project number "PRJNA323784") with ACCESSION MABH00000000. *R. johrii* has an Average Nucleotide Identity (ANI) of 94.77% to *R. sphaeroides* 2.4.1, *In-silico* DNA–DNA hybridization with *R. sphaeroides* 2.4.1 is 59.3%. Features annotated are genome size is 4,512,111 bp, G+C content is 69.1%, Genes (total) is 4,333, CDS (total) is 4,269, Genes (coding) is 4,035, CDS (coding) is 4,035, Genes (RNA) is 64, tRNAs is 53, ncRNAs is 3, Pseudo Genes (total) is 234.

Poster

Characterization of the bacterial pigments of *Flectobacillus rhizosphaerae* JC isolated from the rhizosphere soil of the *Oryza sativa*

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Pigments are light absorbing compounds that are responsible for the colour that the organism displays. Pigmentation is a characteristic that is common to many species of the bacteria. Diverse groups of pigments are produced by the bacterial domain and they play an important role in their survival. Many physiological processes are dependent on them like photosynthesis, UV protection and anti-oxidant activities. Pigments are nothing but the secondary metabolites which have incredible potential in the field of medicine. Cyanobacteria have phycobilin pigments for the photosynthesis. Other examples include *Serratia marcescens* that produces prodigiosin. *Streptomyces coelicolor* (prodigiosin and actinorhodin), *Chromobacterium violaceum* (violacein) and *Thialkalivibrio versutus* (natronochrome and chloronatronochrome). Moreover, they are used as an alternative to the synthetic ones due to their better degradability and higher compatibility with the environment. *Flectobacillus rhizosphaerae* , are one of the many interesting coloured bacterias. *Flectobacillus rhizosphaerae* is isolated from the rhizosphere soil sample of *Oryza sativa* .It is a gram-negative bacteria. They produce pale light orange coloured pigments. Characterizing pigments and their corresponding analogues were the main challenges of our work. *Flectobacillus rhizosphaerae* , newly isolated bacteria from the lab had uncharacterised pigments. HPLC chromatogram of the extract showed that the absorption spectrum belonged to the region between 450 to 500 nm, characteristic to that of the carotenoid pigments. Pigments were extracted, separated and purified using the column chromatography and TLC. HPLC and LC-MS were done for the whole cell extract. Later, FTIR and LC-MS data led to the general conclusion that these extracted and purified pigments could be novel carotenoids.

Poster

Heterologous expression and *in-silico* analysis of carboxysome protein CcmM of an early diverging cyanobacterium *Gloeobacter violaceus* PCC 7421

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The importance of the carbon dioxide concentrating mechanism (CCM) proteins is elucidated by their potential to augment photosynthetic efficiency of the organisms devoid of them. Cyanobacterial carboxysomes aptly exemplify CCMs, and any information on their constituent proteins would go a long way in achieving the above mentioned objective. The present article reports the study of CcmM, a β-carboxysome lumen protein from an early diverging cyanobacterium, *Gloeobacter violaceus* PCC 7421. The CcmM protein of *G. violaceus* was successfully expressed in *Escherichia coli* where it yielded two polypeptides of approximately 72 kDa and 50 kDa molecular weight. Discovery of internal ribosome entry sites suggested a multi-partite gene encoding more than one forms of protein. The 3D structure of the short form of the CcmM protein namely CcmM-M50, containing RuBisCO SSU repeats was generated *in-silico*. Further, the sequence similarity to γ-carbonic anhydrase (CA) and the complete absence of carboxysomal CA suggest the CcmM of *G. violaceus* to be a functional CA. The increase in the RuBisCO small sub-unit repeats in the recently evolved cyanobacteria in comparison to the early ones and the variance in degree of correlation in evolution of N and C terminals of the protein suggests greater evolutionary potential of CcmM protein.

Section 1.4: Photosystem II and Water Oxidation Mechanism

Lecture

FTIR study on the redox property of the primary quinone Q_A in photosystem II

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It has been known that inactivation of the Mn_4CaO_5 cluster by depletion of Mn and/or Ca ions from photosystem II (PSII) causes the upshift of the redox potential of the primary quinone Q_A, $E_m(Q_A^-/Q_A)$, thus resulting in possible suppression of the electron transfer from Q_A to the secondary quinone Q_B [1]. In contrast, we recently revealed that Mn depletion hardly changed the E_m of Q_B [2]. However, the previous measurements of $E_m(Q_A^-/Q_A)$ using the fluorescence method indirectly monitor the redox state of Q_A, and hence a caution is necessary in interpretation of the data. In this study, we reinvestigated the influence of Mn depletion on $E_m(Q_A^-/Q_A)$ by employing light-induced FTIR difference spectroscopy, which can directly monitor the redox reactions of Q_A and Q_B, and was used for the previous $E_m(Q_B^-/Q_B)$ measurements [2]. Light-induced FTIR difference spectra upon Q_A reduction were obtained with intact and Mn-depleted PSII equilibrated at various electrode potentials. From the potential dependence of the signal intensity, the $E_m(Q_A^-/Q_A)$ of intact PSII was determined to be −100 mV, in agreement with that obtained by the fluorescence method. However, Mn depletion little affected the $E_m(Q_A^-/Q_A)$ value. The effect of Mn depletion on the interactions of Q_A in the PSII proteins was further investigated using light-induced ATR-FTIR spectroscopy [3]. It was shown that Mn depletion little changed a Q_A^-/Q_A difference spectrum, suggesting that the H-bond interaction of Q_A with its surroundings, which should influence the $E_m(Q_A^-/Q_A)$, was virtually unchanged by Mn depletion [3]. It is thus concluded that the large $E_m(Q_A^-/Q_A)$ upshift upon Mn depletion found in the previous works could arise from an unknown effect of Mn depletion in fluorescence detection.

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Lecture

Functional diversity of cyanobacterial D1 proteins

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Due to strongly oxidative chemistry of photosystem II (PSII) water splitting, the D1 protein is prone to constant photodamage requiring its replacement in order to regain PSII function. Cyanobacteria developed multiple strategies to cope with this function loss including the development of a small gene family encoding the D1 protein subunit of photosystem II. Over the years several different forms of D1 proteins were described based mostly on functional studies and recently new variants of this important protein were postulated based on available sequences. There are ample studies dealing with the adaptation of PSII function to different light regimes but not much is known about the ability of these photosynthetic organisms to deal with the lack of photosynthetically active radiation. The main objective of our study was to investigate the changes photosystem II donor and acceptor side function in *Cyanothece sp.* ATCC 51142 during a 12/12 hours light and dark cycles in an effort to understand the intrinsic mechanisms of adaptation to these conditions. We used primarily the measurement of flash-induced chlorophyll fluorescence decay to investigate the function of cyanobacterial PSII in *Cyanothece sp.* ATCC 51142. Using specific electron transport inhibitors we can measure the function of both donor and acceptor side of PSII. Our investigation showed significant differences in PSII function between dark and light periods, both on donor and acceptor side of the system. These differences are also dependent on the cyanobacterial species studied and relate probably to the type of habitat there organisms are adapted to. Our conclusion is that specific cyanobacteria use modification of PSII function during light and dark periods of time as a way to adapt to specific environmental conditions.

Poster

Effect of site-directed mutagenesis of amino-acid residues in cytochrome b_{559} interacting with a phosphatidylglycerol on the function of photosystem II

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Recent X-ray crystallographic analysis revealed that 5-phosphatidylglycerol (PG) molecules per monomer are bound to photosystem II (PSII) dimer. We previously investigated the function of PG in PSII with a *pgsA* mutant defective in the biosynthesis of PG and site-directed mutants of amino acid residues in D1 protein interacting with PG molecules, and found that PG plays important roles in PSII. However, we have not clarified the function of the PG molecule located near the Q_B binding-site.

In this study, we have mutagenized amino acid residues of a-subunit of cytochrome (Cyt) b_{559} interacting with a PG molecule by site-directed mutagenesis in *Synechocystis* sp. PCC 6803. Two amino acid residues (Thr-5 and Ser-11) interacting with the PG molecule are located in the vicinity of Q_B binding-site. Growth rates and photosynthetic activities of the site-directed mutants decreased compared to the control strain. In addition, PSII activities of the mutant cells were largely decreased by exogenous supplementation of artificial quinones. We determined by lipid analysis that approximately one PG molecule per PSII monomer was lost in PSII complexes purified from the mutants. Moreover, binding of extrinsic proteins, PsbV and PsbU to PSII core complex was unstable in the mutant PSII. These results suggest that the PG molecule has important roles in stabilizing structure around the Q_B-binding site and binding of extrinsic proteins.

Poster

Photolytic decomposition of water by photochemically formed biomimetic photoautotrophic supramolecular assemblies "Jeewanu", synthesised in sunlight exposed sterilised aqueous mixture of some inorganic and organic substances

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Sunlight exposed sterilised aqueous mixture of ammonium molybdate, di-ammonium hydrogen phosphate, biological minerals and formaldehyde shows photochemical formation of abiogenic photoautotrophic biomimetic, self-sustaining supramolecular assemblies "Jeewanu". The photochemical formation of various molecules of biological interest (viz. amino acids in free as well as in peptide combination, nucleic acid bases, sugars, phospholipids have detected [1]. These microstructures have been found to contain ferredoxin-like material in them [2]. Jeewanu have been found to catalyse photolytic decomposition of water utilising sunlight as a source of energy. Further studies using D_2O have shown that hydrogen thus released in the mixture is used in the photochemical fixation of CO_2 and N_2. can catalyse photolytic decomposition of water utilizing sunlight as a source of energy. These findings have been confirmed by using C^{14}, N^{15} [3]. The irradiated sterilized aqueous mixture of inorganic and organic substances shows photosynergistic collaboration of non-linear processes at mesoscopic level leads to emergence of photoautrophic supramolecular assemblies, "Jeewanu" having a double walled boundary for charge separation and having ferredoxin-like material for electron transfer reactions are capable of showing energy transduction in the laboratory simulated atmosphere.

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Poster

Photosystem II/nanosized manganese oxide composite for water oxidation

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Artificial photosynthetic systems replicate the natural process of photosynthesis to capture and store the energy from sunlight [1]. The water-oxidizing site in natural systems is a Mn-oxido cluster hosted by residues of proteins of Photosystem II [2–4]. Herein, Photosystem II/nanosized manganese oxide composite was synthesized and characterized with scanning electron microscopy, transmission electron microscopy, UV-Visible spectroscopy, Fourier transform infrared spectroscopy, X-ray absorption near edge structure, extended X-ray absorption fine structure, X-ray diffraction spectrometry and some electrochemical methods. These results indicated the presence of mixed (III, IV) valence state in the composite. This aspect is further confirmed by the main edge of composite.

Under electrochemical conditions, the peaks attributed to Mn(II)/(III), Mn(III)/(IV) and Mn(II)/(IV) were assigned and compared with other manganese oxides. Linear sweep voltammetry shows that water electro-oxidation occurs at 80 mV less than for Photosystem II without addition of the Mn oxide-based cluster. Thus, Mn oxide maintains its water-oxidizing activity under these conditions. The composite is a new type of structural and functional model for the water-oxidizing complex in Photosystem II.

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Poster

Redox property changes of cytochrome b_{559} of site-directed mutants in Photosystem II complex

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Cyt b_{559} is the peripheral heme protein complex in Photosystem II (PSII), which the heme-Fe ligates to PsbE/His23 and PsbF/His24. We have reported that a heme-ligand mutant of *Thermosynechococcus elongatus* was sensitive for high light conditions [1]. In this study, we investigated further redox properties of Cyt b_{559} of some site-directed mutants containing direct heme-ligand mutants and around heme-ligand mutants.

In the reduced-*minus*-oxidized difference absorption spectra using several mediators, high potential (HP) form (E_m ~330 mV) and intermediate potential (IP) form (E_m ~210 mV) were approximately 80% and 20% in wild-type PSII, respectively. In the around-heme mutant PSII, although the ratio of HP to IP was very similar to that of wild-type PSII, the spectra were broader than those of wild type. These results suggested that this mutation caused structural modifications around heme. In the heme-ligand mutant, we could not obtain any bands in both the difference absorption spectra and the EPR measurements of Cyt b_{559}. These results indicated that this mutant lost oxidation and reduction ability. In addition to the property changes of heme in mutants, we also present that effects of high light conditions on PSII function and proteins.

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Poster

D1' induced changed in PSII electron transport in cyanobacterium *Synechococcus* sp. PCC 7002

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The structure of the photosynthetic machinery in cyanobacteria is highly conserved, as well as in green algae and higher plants. The core proteins of photosystem II (PSII), D1 and D2, bind all the redox-active components involved in electron transfer of PSII. D1 protein is one of the main sites of damage by a variety of environmental factors, requiring its replacement, whereas most of the other PSII subunits remain ordinarily undamaged. The D1 protein family from cyanobacteria contains members with different functionality as an adaptation to different environmental conditions. There are members of the protein family involved in adaptation to high-light conditions, others to UV-B stress and more recently were discovered members induced in low oxygen or micro-aerobic conditions hinting about a role these D1 form play in cellular adaptation to above mentioned conditions.

In this study we used a *Synechococcus* sp. PCC 7002 mutant that has an inactive *psbA* gene, encoding D1' isoform in comparison with the wild type, in order to better understand the role of this D1 protein isoform under a range of environmental factors (UVB, high-light, micro-aerobic conditions). The standard growth conditions for this strain were: light irradiance of 50 μE m^{-2} s^{-1}, and 38°C. During the high-light experiments *Synechococcus* sp. PCC 7002 shows a change in the decay of the fluorescence curve, not seen previously in other species. In our experiments we try to understand the nature and origin of these changes in PSII function in this cyanobacterium in an effort to gain more insight into the mechanisms of cyanobacterial photosynthetic electron transport.

Poster

Spectral characterization of photosystem II reaction center complex isolated from the chlorophyll *d*-dominated cyanobacterium

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Chlorophylls (Chls) play essential roles in energy transfer, charge separation and electron transfer in Photosystem (PS) I and II. *Acaryochloris marina* is a unique cyanobacterium that differs from the majority of photosynthetic organisms by having Chl *d* as the major pigment (>95%) [1]. The absorption peak of Chl *d* is approximately 30 nm longer than that of Chl *a* in organic solvents. Despite of the majority of Chl *d*, Chls *a* were detected in both PS I and II [2, 3]. In our previous analyses, a small number of Chls *a* were bound in PS II complex. If Chl *a* is involved in the charge separation process, our current understanding of the overall energetic of the PS II would need to be modified. In this study, we isolated the PS II reaction center (RC) complex, which is a minimum complex capable of the charge separation, from *A. marina*. We characterized the spectral properties of PS II RC. We will discuss the site energies of Chls in PS II RC.

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Section 1.5: Energy Transfer and Trapping in Photosystems

Lecture

The Ross Sea haptophyte *Phaeocystis antarctica* and dinoflagellate cells hosting kleptoplasts derived from it are both capable of light state transitions

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State transitions (STs) is a reversible physiological process that oxygenic photosynthetic organisms use in order to minimize imbalances in the electronic excitation delivery the reaction centers of Photosystems I and II (PSI, PSII) and to optimize, thus, photosynthesis. STs have been studied extensively in plants, green algae and cyanobacteria, but sparsely in secondary endosymbiosis algae with a red endosymbiont, such as diatoms and haptophytes. In the present work, we examine whether haptophyte *Phaeocystis antarctica*, and dinoflagellate cells that host kleptoplasts derived from *P. antarctica* (RSD), both endemic in Ross Sea, Antarctica, are capable of light adaptive STs. In these organisms, Chl *a* can be excited either directly by light absorption, or indirectly be electronic excitation (EE) transfer from ultraviolet light absorbing mycosporine-like amino acids (MAAs) [1]. Here we show that dark-adapted *P. antarctica* shifts from state 1 (ST1; more EE in PSII) to state 2 (ST2; more EE to PSI) on adaptation to PSII-selective light, as revealed by the spectral distribution of directly-excited Chl *a* fluorescence. The same is true for RSD cells, although to a lesser extent. In contrast, no STs is clearly detected in the case of indirectly excited Chls *a* both in *P. antarctica* and in RSD cells.

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Lecture

Excitation energy transfer processes in plant light harvesting complexes studied by multidimensional electronic spectroscopy

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Light-harvesting antenna systems such as LHCII, the primary light-harvesting complex in plants, are essential for the photosynthetic process that eventually powers the biological world. The excitation energy transfer (EET) processes in light-harvesting systems are therefore of strong interest to scientists.

We performed 3rd order 2D electronic spectroscopy (2DES) on solubilized LHCII trimers to study the the Chl *b* band to the Chl *a* band EET dynamics [1, 2] at room temperature. Using a new technique known as the 5th order 3D electronic spectroscopy (3DES) we further study the multistep EET dynamics from excitons in the Chl *b* band to the low-energy level Chl *a* states via mid-level Chl *a* energy states [3]. We have also use 2DES to study the equilibration dynamics within the Chl *a* band and reveal uphill and downhill energy transfer dynamics between different Chl *a* exciton states [4].

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Poster

Excitation energy transfer and trapping in Photosystem I in solution and immobilized on conducting glass

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Photosystem I (PSI) is a photosynthetic protein-pigment complex, commonly found in plants, algae and cyanobacteria. In these organisms it cooperates with photosystem II (PSII) in the light-driven transfer of electrons from the water molecule to the $NADP^+$ molecule, which is next used to reduce the carbon dioxide into carbohydrates in the light-independent phase of photosynthesis. Pigments form the antenna systems that capture sunlight energy and transfer it to the reaction centers, where it is used to initiate charge separation. Algal and plant PSI are equipped with additional LHCI antenna systems, but with different sizes in both types of organisms. The cyanobacterial PSI does not contain peripheral LHCI antennas, but it exists *in vivo* as a trimer. The monomeric PSI devoid of LHCI antenna is called PSI core and its structure is very similar for all organisms.

We performed time-resolved fluorescence measurements with ~3.5-ps temporal resolution for different PSI preparations: algal PSI core particles and PSI-LHCI complexes in solution as well as monomeric and trimeric forms of cyanobacterial PSI in solution and immobilized on FTO conducting glass. Comparative analysis of the obtained results allowed us to draw some important conclusions on the excitation dynamics in the studied PSI samples: (1) The average decay time of excited states of bulk chlorophylls in PSI core is almost identical in the case of algae and cyanobacteria. It is equal to ~13 ps or ~17 ps for RC in open or closed state, respectively [1, 2]. (2) The effective time of excitation transfer from the LHCI antenna system to the PSI core in algal PSI-LHCI complex is equal to 12–15 ps [1]. (3) The energy threshold for trapping is located around 675 nm and probably defined by the absorption band of the $A–A_0$ pair. (4) Lowering the temperature (to 77 K in our experiment) or immobilization of PSI on conducting glass leads to significant acceleration of trapping in RC [2].

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Poster

Localization and characterization of chlorophylls *f* in photosystem I and II complexes

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In general, photosynthetic organism uses chlorophyll (Chl) *a*, *b*, and *c* as antenna pigments. In 1996, Chl *d* dominated cyanobacterium was found in the pacific island [1]. In 2010, Chl *f* containing cyanobacterium was found in the living stromatolite in western Australia [2]. These two are more red-shifted Chls than Chl *a* in their absorption spectrum. Chl *f* has the structure [2-formyl]-Chl *a,* and is most red-shifted Chl so far. We reported the energy transfer mechanisms in cells and thylakoid membranes [3, 4]. In this study, we isolated the photosystem (PS) I and II complexes from Chl *f* containing cyanobacterium and performed spectroscopic analyses. The photoinduced $P700/P700^+$ spectrum revealed the molecular species of the special pair was consisted of Chl *a*/Chl *a′* dimer. The absorption and fluorescence spectra showed the excitation energy was equilibrated between the Chl *a* and Chl *f*. Therefore, uphill energy transfer was occurred in PS I. In PS II, the delayed fluorescence, which originates from charge recombination, was not observed in Chl *f* region. So, Chls *f* also act as antenna Chl in PS II. Judging from the fluorescence spectra, the localization of Chls *f* were located in CP43 and CP47 in PS II.

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Section 1.6: Photosystem I and Bacterial Photosynthesis

Lecture

The evolutionary pressures for oligomerization of Photosystem I

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Photosystem I (PSI) forms trimeric complexes in most characterized cyanobacteria, yet in plants/algae PSI is monomeric. Recently reports on the tetrameric PSI raised questions and speculations about its occurrence, formation mechanism as well as physiological and evolutionary significance. In this study, by examining PSI in 61 cyanobacteria, we show that tetrameric PSI, correlating with a unique *psaL* gene, is widespread in the heterocyst-forming cyanobacteria and their close relatives. Physiological studies on these cyanobacteria revealed that the formation of tetrameric PSI is favored under high light, with increased relative PSI tetramer abundance, stability, and carotenoids content. These carotenoids include some novel PSI cofactors: myxoxanthophyll, canthaxanthan and echinenone, which putatively play photoprotective roles for PSI. Together this work suggests that tetrameric PSI is an early adaptation to high light and supports the hypothesis of tetrameric PSI being the evolutionary intermediate in the transition from cyanobacterial trimeric PSI to monomeric PSI in plants/algae.

Lecture

Effects of Low pH on Photosystem I

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The increase in 77 K fluorescence of PSI and quenching of PSII fluorescence upon exposure of isolated thylakoid to low pH is not caused by state transitions as evident from the observation that similar change was observed also in the stn7 kinase mutants. On the contrary, the pH induced change in the PSI/PSII ratio was found to be absent in npq4 mutants, providing evidence that PsbS dependent NPQ is involved in regulating energy distribution between the two photosystems. Results from 77 K fluorescence excitation spectra indicated that pH does not affect the attachment of the LHC system with the photosystems as such, but simply enhances the spillover of energy between the two photosystems. An enhanced cyclic electron flow around PSI also supports this contention. Changes in the redox state of Photosystem I (PSI) were studied in spinach leaf discs suspended in buffers of different pH (pH 7.5, 6.5, 5.5 and 4.5). By measuring absorbance changes at 820 nm, it was observed that under normal conditions, the electrons were supplied by Photosystem II (PSII) for the photo-oxidation of P700 while in the presence of DCMU when electrons coming from PSII are blocked, cyclic electron flow (CEF) around PSI was the major source for the absorbance changes observed at 820 nm. This was supported by complete inhibition in the reduction of both single turnover (ST) area and multiple turnover (MT) area, in the presence of DCMU, which is generally filled up by the electrons coming from PSII. In the absence of DCMU, the intersystem electron pool or plastoquinone (PQ) pool was increased at low pH which was probably due to enhanced cyclic electron flow around PSI. Our results also suggest that at low pH, in the absence of DCMU, the major contribution for faster dark re-reduction of $P700^{+}$ is attributed mainly by PSII and CEF PSI while in the presence of DCMU, the significant contribution is provided by CEF PSI and other stromal components.

Lecture

Single-Molecule Spectroscopy study on Photosystem I at low temperatures

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We have conducted the single-molecule spectroscopy of photosystem I (PSI) at liquid nitrogen temperature by using a novel-type cryogenic microscope developed by our laboratory [1]. The study has revealed that a fluorescence intensity from a single PSI often fluctuates on a time scale of seconds. In our experimental condition using a CW HeNe laser as the excitation source, the primary donor P700 repeats the charge-separation and recombination reactions during the measurement. As a result, P700 in the PSI under observation is practically in its oxidized form in average although it is pre-reduced by addition of sodium ascorbate. We interpret the blinking of single PSI as coming from modifications in the energy-transfer pathway between that feeding much exciton to the emitting red Chl and that feeding exciton to the quencher $P700^+$. The energy of the 633-nm photon (absorption) minus the 720-nm photon (emission) is discarded into the complex for each photon absorption. A part of this residual energy may be utilized to induce the conformation changes in the amino-acid residues around chlorophylls, cause the modifications in the site energies of the pigments and the energy-transfer pathway. Surprisingly, we found that the fluorescence-intensity fluctuations of single PSIs were enhanced upon the pre-oxidation of P700 with addition of ferricyanide. In the present experimental condition, the P700 pre-reduced and pre-oxidized PSI are in the redox states of $P700^+A_1^-$ and $P700^+A_1$ during the measurement, respectively. We will discuss the enhanced fluorescence fluctuation in the pre-oxidized sample in association with the different redox states.

1. Yutaka Shibata et al. (2014) *Biochim. Biophys. Acta* 1837, pp. 880-887

Poster

Enhancing electron transfer through *T. elongatus* photosystem I (PSI) by bioengineering the PSI-ferredoxin interface

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Ferredoxin (Fd) shuttles electrons from photosystem I (PSI) to Fd-NADPH reductase (FNR) within the stroma of chloroplasts and cyanobacteria. This role requires delicate balance between affinity for PSI/NFR and diffusion between these two binding partners in the stroma. However, unlike the *in vivo* process, our work on applied photosynthesis does not require a tradeoff between binding affinity and stromal diffusion, with the goal of building a near solid state system with maximum rates of forward electron transfer. The atomic level interaction between Fd and PSI is still unclear and not much is known about the binding partners in this interaction, neither we have a crystal structure of the complex itself. This interaction is crucial to understand the assembly of our model bio-hybrid solar cells. Previously we have computationally shown that Fd interacts with the stromal subunits of PSI (PsaC, PsaD and PsaE) in three different possible conformations of which two of them have Fd in a nearly 180° rotation. Nevertheless, these conformations have highly 'frustrated' regions that are involved in there binding, but the interaction is not tight enough for them to remain as a complex as the biological significance of this interaction is limited to shuttling of reduced Fd from PSI to FNR. For the purpose of building our bio-hybrid solar cells we have engineered Fd with a TiO_2 binding peptide, hence a modified Fd (LSTB1-Fd), attaching Fd *in vitro* to the TiO_2 nanoparticles. Firstly, we have computationally generated single (S63D/E/W and F38A/W) and double mutants (combination of both) of LSTB1-Fd, and have shown enhanced binding interaction with PSI by rigid body directed docking and molecular dynamic simulation. These mutants can potentially lead to an enhanced or 'sticky' interaction between the two proteins. To confirm this, we have experimentally generated these mutants and performed *in vitro* chemical crosslinking assay to investigate the binding profile of these mutants. We also plan to perform back-scattering interferometry and surface plasmon resonance to characterize these mutants for their enhanced binding efficiency.

Poster

High resolution structure of *Synechocystis* Photosystem I

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Oxygenic photosynthesis is the main process that converts light energy from the sun into chemical energy accessible for living organisms. The products of Oxygenic photosynthesis virtually provide all higher life on earth with energy, food and oxygen in the biosphere. This process has a profound impact on the planet's atmosphere and climate. The primary event of this process is light induced charge translocation, catalyzed by multi subunits protein complexes, photosystem I (PSI) and photosystem II (PSII), embedded in the thylakoid membrane of cyanobacteria, algae and plants. The complex of PSI is also known to be the most robust and highly efficient nanophotochemical machine, with internal quantum yield efficiency approaching 100% at room temperature. Photosynthetic studies could not demonstrate a chemical compound that will inhibit its function, so it is interesting how this stability evolved.

Only two high resolution crystal structures of PSI are available so far. The first crystal structure has been determined in 2001 for thermophilic cyanobacterium *Synechococcus elongatus* at 2.5 Å resolution. However thermophilic organisms are niche inhabitants, whereas cyanobacteria in general are probably the most successful group of microorganisma on earth, therefore we embarked on purifying, crystallizing and elucidation the structure of the PSI complex from the mesophilic cyanobacterium *Synechocystis sp.* PCC 6803. This structure will be interesting from the evolutionary point of view, especially when the plant PSI structure at 2.6 Å resolution is known as well.

We succeeded to isolate, purify and obtain the crystal structure of the trimeric PSI complex from a Synechocystis to 2.5 Å resolution. Considering the unusual stability and effective function of the PSI complex, not many changes are expected to happen between those three organisms. Nevertheless this resolution allows us a more detailed comparison of the position aspects of the chlorophylls, carotenoids and cofactor compounds, between the thermophilic and mesophilic photosystem I complexes, which will be discussed.

Poster

Glutathionylated human mitochondrial Mia40 (CHCHD4) is required for cytochrome c stability and complex IV biogenesis

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Human Mia40 (mitochondrial intermembrane space (IMS) protein), is involved in import of IMS proteins from cytosol to IMS. The *in vivo* redox state of Mia40 at steady state is about 70% oxidized and local glutathione pool provides reducing equivalents to Mia40. However, it is not clear whether glutathione directly interacts with Mia40 or Mia40 reduction is mediated by catalyst such as glutaredoxin. In the present study we have identified that Mia40 undergoes post translational modification like glutathionylation. Both *in vitro* and *in vivo* studies confirmed the glutathionylation of hMia40. Further we identified, majorly four cysteines of hMia40 were prone to glutathionylation. However we couldn't find any defect in import of IMS proteins in these cysteine mutants of Mia40. Interestingly, we found the apparent decrease in Cytochrome-C levels in these mutants, suggesting the importance of glutathionylated Mia40 in Cytochrome-C stability. Further michondrial complex IV activity was also decreased in these mutants. Our findings indicate that the glutathionylation of Mia40 could play an important role in cytochrome c dependent complex IV biogenesis of electron transport chain.

Section 1.7: Carbon Fixation (C3 and C4) and Photorespiration

Lecture

Acclimation of C_4 photosynthesis to low light

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C_4 plants include some of the world's most important food, feed and biofuel crops (e.g., maize, sorghum and sugarcane), dominate the understory of warm climate ecosystems, and account for ~20% of terrestrial productivity. During C_4 photosynthesis, the CO_2 concentrating mechanism (CCM) and the inevitable CO_2 leakage out of the bundle sheath require additional energy which may limit the productivity of C_4 plants in low-light environments such as dense canopies and semi-closed woodlands. C_4 photosynthesis evolved independently many times resulting in multiple CCM pathways. However, little is known about how these pathways respond to low light. To address this knowledge gap, we investigated the response of C_4 grasses with different biochemical subtypes (NADP-ME, NAD-ME and PEP-CK) to growth under 100% (control) or 16% (shade) sunlight. The shade treatment reduced plant productivity to a greater extent in NAD-ME and PEP-CK relative to NADP-ME grasses. Photosynthetic carbon isotope discrimination (Δ) and bundle sheath leakiness (ϕ) tended to be lower in shaded NADP-ME plants while photosynthetic quantum yield (Φ_{max}) tended to be lower in shaded NAD-ME plants relative to the other treatments. These changes corresponded with greater reductions of functional photosystem II and leaf absorptance in the NAD-ME species and of cyclic electron flow in the NADP-ME species under shade. The high cyclic electron flow in NADP-ME species under control conditions is associated with enrichment of the NADH dehydrogenase-like (NDH) complex in the thylakoid membrane of the bundle sheath tissue. In conclusion, low light compromised CCM efficiency to a greater extent in NAD-ME and PEP-CK species relative to NADP-ME species. This is an important and novel contribution because our data identified different photosynthetic responses to low light among C_4 grasses depending on the biochemical subtype. The outcomes have important implications for modelling the productivity of C_4-dominated ecosystems and for improving light use efficiency in C_4 crops.

Lecture

Is Photosynthetic behaviour of plants different at High Altitude?

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Persistent low temperature, large diurnal temperature difference, high radiation load, and low partial pressure of CO_2 are some of the challenging conditions for optimal plant growth at high altitude (HA), as compared to those at low elevation. While photosynthetic adaptation to low temperature and changing light regimes has adequately been studied, role of reduced partial pressure of CO_2 affecting photosynthetic metabolism has less extensively been addressed. Over last half a century, numerous workers have reported various morphological, physiological, and biochemical adjustments in plants with change in elevation, that could be of adaptive significance. We reported that insensitivity of stomata to light, higher efficiency of CO_2 uptake, and higher stomatal conductance helps photosynthesis in the environment at HA. We further reported enhanced activities of enzymes phosphoenolpyruvate carboxylase (PEPCase), aspartate aminotransferase (AspAT) and glutamine synthetase (GS), coupled with variation in the primary product of photosynthesis, and suggested a shift in photosynthetic metabolism in C_3 plants at HA with its possible significance in conservation of carbon and nitrogen at HA. Later, we showed a similar enzymatic shift for same species (*Rumex nepalensis* and *Trifolium repens*) in plants grown under low CO_2 (250 ± 10 µmol mol^{-1}) to show that low partial pressure of CO_2 could be an important stimulus for photosynthetic acclimation at HA. Over-expression of genes for these three enzymes in *Arabidopsis thaliana* suggested recapture of photorespiratory CO_2 with concomitant increase in photosynthetic rate, higher shoot biomass and seed yield in comparison to the wild-type (WT) plants.

Lecture

Transient expression and localization of a cyanobacterial bicarbonate transporter BicA into chloroplast of *Nicotiana benthamiana*

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The incorporation of a cyanobacterial bicarbonate transporter such as BicA to inner chloroplast envelope of C3 plants is believed to be effective in improvement of CO_2 fixation. In this study, data is presented on generating minimal chimeric construct/s containing *bicA* gene from *Synechococcus* sp. PCC 7002 and its subsequent targeting into the chloroplasts of *Nicotiana benthamiana* through nuclear transformation. To accomplish the intended targeting and expression in the plant system, specific cleavable transit peptide (TP) sequences were taken from N-terminus of chloroplast inner envelope proteins of *Arabidopsis thaliana* i.e., Inner translocon complex or TIC55 transporter (AT2G24820) and maltose transporter (AT5G17520), named as TICTP and MEXTP respectively. Chimeric genetic constructs were synthesized by fusing TP sequences and *bicA* gene (TICTP/MEXTP+*bicA* gene) which being huge in size (approximately 1.85 kb), was first ligated into an intermediate vector *viz.* pCold-IV and finally cloned into the plant expression vector i.e., pCAMBIA-1302 upstream of the mGFP5 reporter gene. To study the transient expression and to determine the intended localization of the targeted protein, the above genetic constructs were transformed into *N. benthamiana* via *Agrobacterium* mediated agroinfiltration method. The expression of these GFP tagged fusion proteins (BicA+mgfp5) was examined in transiently transformed leaves. The incorporation of the targeted gene in plant system was checked at DNA and mRNA levels by performing diagnostic PCRs using specific primers for TP sequences, *bicA* gene and *mgfp5* gene while protein expression was checked by western blotting using anti-GFP antibodies. The successful amplification of these sequences and the presence of expected sized protein bands in western blot confirmed the expression of both the constructs (TICTP-*bicA-mgfp5* and MEXTP-*bicA-mgfp5*) in the transformed leaves. Further, the localization of fusion proteins was visualized in protoplast cells by confocal laser scanning microscopy which indicated their presence in the chloroplast. This study is an important step towards achieving the long term objective of plant productivity enhancement by manipulating the C3 plant machinery.

Lecture

Posttranslational modifications in *Chlamydomonas* Rubisco influence catalysis

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In the 1.4-angstrom x-ray crystal structure of *Chlamydomonas* Rubisco, four previously unknown posttranslational modifications were observed in the chloroplast-encoded large subunit. Pro-104 and 151, which are conserved in all Rubisco enzymes, are hydroxylated, and Cys-256 and 369, which are replaced by Phe and Val in land plants, are methylated. Because algal Rubisco has a higher rate of carboxylation but lower CO_2/O_2 specificity than land-plant Rubisco, the posttranslational modifications might contribute to these differences. Directed mutagenesis and chloroplast transformation of Chlamydomonas were used to test the essentiality of the modified residues by replacing each with Ala. The Cys residues were also replaced with the residues most often found in land-plant Rubisco (Phe and Val). The single-mutant (P104A, P151A, C256A, C369A) and double-mutant (P104A/P151A and C256A/C369A) Ala-substituted strains grow photosynthetically at 25°C, but the C256A and C256A/C369A mutants have decreased levels of holoenzyme and cannot grow photosynthetically at 35°C. Biochemical analysis has revealed a variety of alterations in kinetic constants, and the P104A/P151A, C256A, C369A, and C256A/C369A enzymes have decreases in CO_2/O_2 specificity. Thus, the modified residues are not essential for Rubisco function, but they appear to influence catalysis even though they are far from the large-subunit active site. The phylogenetic C256F, C369V, and C256F/C369V mutants also grow photosynthetically, but their Rubisco enzymes have normal CO_2/O_2 specificity values. Posttranslational modification may regulate Rubisco function.

Lecture

Importance of dark respiration in optimizing photosynthetic performance of plants – a turn of the role from in-significance to significance: A personal perspective. Dedicated to Prof. A.S. Raghavendra

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In higher plants, chloroplastidic photosynthesis, a major interest of photo-physiology was thought to be independent from dark respiration which occurs in its sister organelle, mitochondria of photosynthetic tissue. The role of dark respiration in sustaining photosynthesis was a null hypothesis until 1980's and therefore scientific reports questioning the positive role of dark respiration in photo-physiology were none/scanty till then.

In late 1980's, Prof. A. S. Raghavendra postulated and demonstrated that exposure of green leaf tissues to short cycles of light and dark periods, in lieu of continuous illumination, would not only help in sustenance but also enhanced the photosynthetic performance by several folds. This hypothesis was extensively evaluated in his laboratory using *in vitro* systems of isolated mesophyll protoplasts as well as excised leaf discs of *Pisum sativum*, eventually, leading to a major concept "Mitochondrial respiration optimize chloroplastic photosynthesis by oxidizing excess chloroplastic reducing equivalents through operation of specific redox metabolite shuttles". The beneficial effect of dark respiration was highly pronounced under abiotic stress conditions such as light, osmotic (water) and temperature. Isolated mesophyll protoplasts, due to their ease of manipulation enabled the use of metabolic inhibitors and facilitated the studies of interaction between chloroplasts and mitochondria by simple and economical way of measuring oxygen using polarographic method. The studies extended in this direction using knock-out plants and -omics technology revealed the importance of *AtAOX1a*, ROS, antioxidant system and other molecular mechanisms underlying the beneficial interactions between these organelles. Further, the significance of *AtAOX1a* in preventing oxidative damage and maintaining redox homeostasis was perceived using yeast as a model organism. In summary, pursuance in this area of research opened up the highly complex and dynamic nature of these inter-organelle interactions in order to keep up with each other's ever changing demand and burden, which can be a direct measure of the extent of the stress exposed.

Lecture

Towards C4 rice: Overexpression of phosphoenolpyruvate carboxylase, phosphoenolpuruvate carboxy kinase and carbonic anhydrase in *Arabidopsis thaliana* enhances its photosynthesis, productivity and water use efficiency

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To meet the challenge of growing population in India food production need to be increased. Mostly, C3 plants are underachievers. Plants with C4 photosynthesis are not only efficient in carbon assimilation, but they also have an advantage under unusual growth conditions. In C4 photosynthesis, the primary CO_2 fixation is catalyzed by phosphoenolpyruvate carboxylase (PEPC). It is show that overexpression of *Zea mays* (Zm) PEPC cDNA, under the control of ^{35}S promoter, in *Arabidopsis thaliana* resulted in higher ZmPEPC gene expression, ~7–10-fold higher protein abundance and ~7–11-fold increase in PEPC activity in the transgenic lines than that in the vector control. Further, the PEPC overexpressed transgenic plants had higher chlorophyll content, enhanced electron transport rate (ETR), lower non-photochemical quenching (NPQ) of chlorophyll (Chl) *a* fluorescence, and a higher performance index than the vector control. Consistent with these observations, the rate of CO_2 assimilation, the starch content and the dry weight of PEPC overexpressed plants increased by 14–18%, 10–18% and 6.5–16% respectively. We have also overexpressed both PEP Carboxylase and PEP Carboxykinase in *Arabidopsis thaliana* to have increased CO_2 concentration in the vicinity of Rubisco. Overexpression of these enzymes leads to higher electron transport, carbon assimilation, increased biomass coupled with better water use efficiency. Carbonic anhydrase (CA) catalyzes the inter-conversion of CO_2 and bicarbonate used by the primary carboxylating enzymes of C3 and C4 plants respectively. In this study cytosolic carbonic anhydrase (b-CA3) of the C4 dicot *Flaveria bidentis* was overexpressed in C3 *Arabidopsis thaliana* to enhance its photosynthetic efficiency. Overexpression of b-CA3 cDNA resulted in ~2-fold higher CA protein abundance and ~50–65% increase in CA activity. Phosphoenolpyruvate carboxylase (PEPC) plays an anaplerotic role of replenishing the tricarboxylic acid cycle with certain intermediates to meet the demand of carbon skeletons for the synthesis of organic acids and amino acids in C3 plants. Due to higher concentration of HCO_3^- in CA overexpressors, their PEPC activity increased generating more oxaloacetic acid and amino acids. Consequently, their total protein content increased resulting in higher Chl synthesis. Due to their increased chlorophyll and protein content the transgenics had enhanced ETR and lower NPQ of chlorophyll *a* fluorescence. Therefore, CO_2 assimilation, starch content, plant fresh weight and dry weight increased by 10–20% in CA overexpressors. Transgenic plants had lower stomatal conductance, reduce transpiration rate and higher water use efficiency. These approaches are being replicated in rice (*Oryza sativa*) to have increased photosynthesis, plant productivity and grain yield.

Poster

Effect of oxidative and photooxidative stress on photorespiratory metabolism in leaf discs of pea, *Pisum sativum*

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In plant cells, photorespiration has a crucial role in keeping the reactive oxygen species (ROS) levels low and maintaining the redox homeostasis. When the plants are exposed to stress conditions such as high light or drought, the levels of ROS increase. Chloroplasts, peroxisomes and mitochondria are important sources of ROS. We have examined the effect of oxidative stress, induced in mitochondria leading to an increase in ROS, on the patterns of key photorespiratory enzymes localized in chloroplasts or peroxisomes. We used menadione, a redox active quinone, which interferes with mitochondrial electron transport to generate ROS. ABA or riboflavin were used as comparison to induce oxidative stress. Experiments were performed in dark, normal (300 µE) or high light (1200 µE) in leaf discs of pea. The photorespiratory enzymes, chosen were glycolate oxidae (GO), catalase (CAT) and hydroxypyruvate reductase (HPR) (localized in peroxisomes) and glycerate kinase (in chloroplasts). Aconitase in mitochondria and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in cytosol were studied for comparison. The activities of GO and catalase increased upto 50%, while the activity of HPR had only a marginal increase in menadione treated samples under high light. Mitochondrial aconitase and cytosolic GAPDH activities decreased on exposure to menadione. Chloroplastic glycerate kinase activity was increased in presence of menadione and high light. Not only the activity, but also protein levels of GO and catalase were increased in menadione treated leaves under high light. Our results indicated that oxidative stress induced by menadione, modulates markedly key photorespiratory enzymes, particularly in high light. The enzymes located in mitochondria (e.g. aconitase) or cytosol (GAPDH) did not respond as strongly as those in peroxisomes. We suggest that the oxidative stress, originating in mitochondria can affect photorespiratory components in peroxisomes as well as chloroplasts. Further experiments would focus on transcript and metabolite analysis during oxidative and photooxidative stress.

Poster

Diurnal changes titratable acidity new CAM plant, *Sedum caucasicum* leaves

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Crassulacean acid metabolism (CAM) is a specialized mode of photosynthesis that features nocturnal CO_2 uptake, facilitates increased water-use efficiency. A large variation in the CAM has been found within the genus *Sedum*. C3 species, CAM constitutive species and CAM inducible species upon water or salinity have been recognized. Results of our initial studies show that diurnal acidity cycle in *Sedum caucasicum* species which is considered endemic in Caucauses occurs similar to that of in CAM plants nevertheless it is grown in green house or naturally. *Sedum caucasicum* species naturally inhabiting sun exposed rocky outcrops. Total titratable acidity in flowers and green stems under natural conditions was less than in leaves and it did not change pronouncedly during the day. Despite the fact that, the quantity of titratable acidity was less in juvenile leaves compared with young and middle-aged leaves of the plants studied under both natural and controlled conditions, cyclic changes of acidity occurred in juvenile leaves contrary to flowers and green stems young and middle-aged ones. Acidity of old leaves, completed their vegetation was relatively less than that of young and middle-aged leaves. It suggests that the rate of photosynthesis and the intensity of metabolism are positively correlated with the total acidity. The obtained results confirm that *Sedum caucasicum* is an obligate CAM plant. Negative correlation is observed between the amount of titratable acidity in the plants subjected to drought and RWC in leaves. As well as in response to environmental temperature and light intensities daily fluctuation of titratable acidity is observed.

Poster

Optimizing biocatalytic conversion of carbon dioxide to polyhydroxyalkanoates through self sustained photosynthesis

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Photosynthetic bacteria are natural CO_2 sequesters along with the ability to produce various biobased products. Polyhydroxyalkanoates (PHA) are intracellular storage molecules accumulated under carbon and energy stress. This study focuses on producing PHA using lab isolated photosynthetic bacteria with CO_2 as the sole carbon source. Optimizations of growth media and operational parameters are performed to improve the CO_2 capture efficiency and biopolymer production. The experiment was conducted in two phases methodology, with initial growth phase (GP) followed by nutrient starvation phase (NSP) which enables maximum biomass production and PHA accumulation. Substrate availability in both phases of operation showed influence on PHA and biomass production. PHA production of 12% and 20.5% was observed in GP and NSP with the biomass production of 0.35 g/l in GP and 0.8 g/l in NSP. Bacterial chlorophyll (BChl) concentration of 0.38 µg/ml and 0.84 µg/ml was observed in GP and NSP respectively. Carbon uptake by photosynthetic bacteria in both the phases was analyzed and found to be 50% and 35% in GP and NSP respectively. The extracted PHA was characterized by FT-IR which revealed the presence of different conformational bands representing the presence of intracellular polymer PHA. This study enables the sustainable conversion of CO_2 to biocommodities.

Poster

Overexpression of *Zea mays* phosphoenolpyruvate carboxylase and malate dehydrogenase in *Arabidopsis thaliana* to enhance photosynthesis and tolerance to abiotic stress

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Plants with C4 photosynthesis are efficient in carbon assimilation and have an advantage under unusual growth conditions. In C4 photosynthesis, the primary CO_2 fixation is catalyzed by phosphoenolpyruvate carboxylase (PEPC) that converts PEP to a 4-carbon compound oxaloacetic acid. The plastidic NADP-malate dehydrogenase (*ZmNADP-MDH*) is responsible for the catalysis of oxaloacetate to malate. We have overexpressed individually cDNA of C4 pathway enzyme cytosolic *Zea mays PEPC* and plastidic *ZmNADP-MDH* in a model plant *Arabidopsis thaliana* under the control of ^{35}S CaMV promoter to achieve higher photosynthetic efficiency in C3 plants and tolerance to salinity. Overexpression of PEPC played an anaplerotic role to increase the supply of 4-carbon carboxylic acids, which provided carbon skeletons for increased amino acid and protein synthesis. Higher protein content must have been responsible for increased metabolic processes including chlorophyll biosynthesis, photosynthesis, and respiration. Consequently, the PEPC-overexpressed transgenic plants had higher chlorophyll content, enhanced electron transport rate (ETR), lower non-photochemical quenching (NPQ) of chlorophyll *a* fluorescence, and higher performance index (PI) than the vector control. Consistent with these observations, the rate of CO_2 assimilation, the starch content, and the dry weight of PEPC-overexpressed plants increased by 14–18%, 10–18%, and 6.5–16%, respectively. Unlike PEPC overexpressors the transgenics of *A. thaliana* overexpressing *ZmNADP-MDH* had similar chlorophyll, carotenoid and protein content as that of vector control. Their photosynthetic electron transport rates, carbon assimilation rate and consequently, fresh weight and dry weight were almost similar to vector controls. However, both the transgenics, i.e. PEPC- and NADP-MDH- overexpressors were tolerant to salt stress (150 mM NaCl) as they had higher variable to maximum Chl *a* fluorescence (F_v/F_m) ratio, higher PI, higher ETR, and lower NPQ than the salt-treated vector controls. However their mechanisms of tolerance to salt stress were different. PEPC overexpressors were tolerant to salt stress as they had increased ability to synthesize amino acids, including the osmolyte proline. The tolerance of *NADP-MDH* overexpressors to salt stress was due to operation of an efficient malate valve that plays a major role in maintaining the cellular redox environment. These results demonstrate that expression of cytosolic C4 carboxylating enzyme PEPC and plastidic NADP-MDH in a C3 plant may increase its photosynthetic potential and confer tolerance to salt stress.

Poster

Metabolic fate of C_3 and C_4 crop under projected levels of tropospheric O_3 and CO_2: an insight to its physiology and metabolomics

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The present study was conducted to understand variation in physiological responses of C_3 and C_4 crop, fate of carbon towards primary and secondary metabolites and gene expression of antioxidative enzymes at elevated CO_2 and O_3. Treatment consists of ambient CO_2+ambient O_3 (ACO_2 + AO_3) as a control, elevated O_3 (EO_3), elevated CO_2 +ambient O_3 (ECO_2), and elevated CO_2 + elevated O_3 (ECO_2 + EO_3). The elevated concentration of O_3 (ambient (46.13) + 20 ± 5 ppb) and CO_2 (570 ± 25 ppm) were selected to match with the predicted concentration in the end of the century under A1B Scenario AR4 of IPCC. Photosynthesis rate decreased in EO_3 and increased in ECO_2 and ECO_2+EO_3 in C_3 crop and in C_4 crop no variation was recorded in ECO_2 and increase in ECO_2+EO_3.Stomatal conductance was higher in EO_3 followed by ECO_2 and lower in ECO_2+EO_3 compared to control in both the crops. SDS-PAGE result showed more degradation of large subunit of RUBISCO under EO_3. Variations in its Photosynthetic Nitrogen use efficiency (PNUE) and water use efficiency (WUE) was recorded in C3 and C4 crop under different treatments of elevated CO_2 and O_3. In C_3 crop higher ABA content was observed while in C_4 crop higher SA content was recorded. Results of amino acid profiling showed higher amino acid in ECO_2+EO_3 in C_4 crop and reduction in ECO_2 treatment in C_3 crop. Characterization of polyphenol showed presence of Apigenin derivatives, Chysoeriol derivative, Tricetin derivatives and Luteolin derivative in C_3 and C_4 crops and many unknown compounds of C_4 crop. These compounds were higher in EO_3 treatment in C_3 crop and ECO_2 treatment in C_4 crop. Results of sugars and starch showed more diversion of higher accumulation in ECO_2 and reductions in EO_3. The present study concludes that pearl millet is a highly tolerant C_4 crop under EO_3 and C_3 crop will be more benefitted under elevated CO_2 and has more potential to ameliorate the deleterious effects of present and future levels of O_3.

Poster

Impact of elevated carbon dioxide on photosynthesis and productivity in response to climate change

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Atmospheric CO_2 concentration has risen at an accelerating pace since the start of the industrial revolution because of burning of fossil fuel and deforestation. Prior to the industrial revolution [CO_2] was stable at about 270 ppm; today [CO_2] is increased to approximately 400 ppm, and by the middle of this century it is predicted to reach 500 ppm and by the end of the century the CO_2 concentration is likely to reach 650 ppm. Today's crop and natural vegetation are growing at an elevated [CO_2] level that has not been experienced by terrestrial or aquatic vegetation for past thousands of years. Understanding how plants respond and might be adapted to a future increase in [CO_2] will also help us understand how they are currently responding and how they may have adapted to the increase that has already occurred. Mustard (*Brassica juncea* L.) is an important oil seed crop that is widely grown in India. Therefore, the impact of elevated [CO_2] (585 µmol mol^{-1}) on pigment and protein content, chlorophyll a fluorescence, photosynthetic electron transport reactions, CO_2 assimilation, biomass production and seed yield potential was ascertained in *Brassica juncea* cv Pusa Bold, an important oil seed crop of India, grown inside free air carbon dioxide enrichment (FACE) rings installed in Jawaharlal Nehru University campus, New Delhi, (28°32'24"N 77°10'2"E). Mustards plants were grown for 3 consecutive winter seasons i.e., 2010-2013 in ambient or elevated [CO_2] (585 µmol mol^{-1}), in open field conditions. Elevated [CO_2] had no significant effect on the minimal fluorescence (F_0), while quantum efficiency (F_v/F_m) slightly increased. Electron transport rate, photosystem I, photosystem II and whole chain electron transport rates partially increased in elevated [CO_2]. However, the net photosynthesis rate (An) increased by ≈45% in 3 growing seasons in elevated [CO_2] primarily due to a reduction in photorespiration as the ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) carboxylation reaction is favoured in these conditions. The stomatal conductance (g_s) and transpiration rate decreased resulting in higher photosynthetic water use efficiency. The photosynthesizing surface i.e., number of leaves per plant and leaf area index substantially increased leading to higher biomass and seed yield in elevated [CO_2]. Our genomics experiments revealed that most of the photosynthetic and respiratory genes were differentially regulated in high CO_2. Acclimatary downregulation of photosynthesis and plant productivity was not observed in 3 consecutive growing years suggesting that in the absence of any kind of nutrient limitation, *Brassica juncea* is highly responsive to elevated CO_2 whose yield potential shall increase in changing climatic conditions.

Poster

Carbon and nitrogen isotope labeling studies and photosynthetic enzymes' activity determinations in switchgrass cultivars

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Switchgrass (*Panicum variegatum* L) is among the species selected by US-Department of Energy (DOE) for development as a bioenergy crop, to accelerate a sustainable production of cellulosic ethanol. Populations of switchgrass occur as lowland and upland ecotypes with divergent growth habits and yield potential. Selection and sustainably growing switchgrass requires a comprehensive understanding of its primary and secondary metabolism of its various cultivars. Previous studies have already established that Swtichgrass is a C_4 plant with NAD-ME photosynthetic pathway type. The present study is however focused on characterizing a few of the field grown Switchgrass cultivars for their photosynthetic characteristics and their stable carbon isotope ratios. Cultivars of switch grass showed: a) a range in activity of both phosphoenolpyruvate carboxylase (67.1–231.4 μmol mg^{-1} protein) and NAD-malic enzyme (220–317 μmol mg^{-1} protein) and b) a variation in their carbon isotope ratio values (12.4 to 13.3‰). Data will also be presented on enzyme activities and levels, results from field labeling of switchgrass cultivars with stable isotopes, and changes in transcript abundances of protein kinases and protein phosphatases in switchgrass leaves.

Poster

Modulation of PR *versus* CCM gene transcript levels in *Chlamydomonas reinhardtii* during light-dark diurnal cycles, circadian free-flow conditions and mixotrophy

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Carbon Concentrating Mechanism (CCM) and Photorespiration (PR) are interlinked and co-regulated in *Chlamydomonas reinhardtii*, but the conditions where the co-regulation changes are not sufficiently well explored. We have investigated transcript level changes of CO_2-concentrating mechanism (CCM) and Photorespiration (PR) genes during light–dark (12 h:12 h) cycles in synchronized *Chlamydomonas reinhardtii* at air-level CO_2. CCM and PR gene transcript levels vary at various times of light–dark cycles, even at same air-level CO_2. In CCM, Transcripts of inorganic carbon transporter genes (HLA3, LCI1, CCP1, CCP2 and LCIA) and mitochondrial carbonic anhydrase genes (CAH4 and CAH5) are up regulated in light, following which their levels decline in dark. Contrastingly, transcripts of chloroplast carbonic anhydrases namely CAH6, CAH3 and LCIB are up regulated in dark. PR transcripts namely GDC, GDCH, PGP1, AAT1 and SGAT are up regulated in dark. Moreover, the up regulation of transcripts in dark was undone by high CO_2, suggesting that the dark induced CCM and PR transcripts were regulated by CO_2 even in dark when CCM and PR are absent. Both CCM and PR transcript rhythmic changes appear not be linked to cellular circadian clock as the 'free-running state" does not retain any discernible rhythmicity In spite of high transcript levels in dark, CAH3 protein reached peak level only in light and localized entirely to pyrenoid, a site functionally relevant for CCM. Moreover, in dark, CAH3 protein level not only reduced but also the protein localized as a diffused pattern in chloroplast. We propose that transcription of most CCM and PR genes, followed by CCM protein level changes including their intracellular localization of a subset is subject to light–dark cycles. Asynchronous continuous light cultures, upon shifting to low from high CO_2 exhibit only transient induction of PR transcripts while CCM transcript induction stays robustly stable, indicative of varying co-regulation of PR *versus* CCM gene transcription. Lastly, we also describe that both CCM and PR transcripts are induced in low CO_2 even in mixotrophic cultures, but only in high light, the same being attenuated in high CO_2, implying that high light is a mandatory "trigger" for CCM and PR induction in mixotrophy. Our study provides comprehensive analyses of conditions where CCM and PR are differently co-regulated, setting a paradigm for more detailed mechanistic probing of these responses.

Section 1.8: Artificial and Applied aspects of Photosynthesis

Lecture

A set-up for studying effects of environmental factors on a photocurrent generated by a solar cell based on titanium dioxide and plant photosensitizers

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Rapid economic development requires significant increase of energy production. Solar energetics is one of the main directions in use alternative energy sources. For the realization of potential capabilities of solar energetics, it is necessary to construct highly-effective converters of solar irradiation to electrical energy. The pigment-protein complexes of plant or bacteria photosynthetic apparatus may be one of the possible alternatives to expensive, complicated and ecologically dangerous in its production semi-conductive photosensitizers used in solar cells (SC). To understand processes occurring in the SCs, in the real conditions of industrial operation it is necessary to study the dependence of the efficiency and stability of the SCs on environmental factors. For laboratory modeling of these conditions, a new set-up has been designed and constructed that makes it possible to study the photocurrent generated by SCs as a function of temperature, intensity, and spectral composition of light. Preliminary results are shown for two types of solar cells with two photosensitizers: thylakoid membrane preparations and anthocyanin-enriched raspberry extracts. It was shown that electrogenic activity decreased by a half at 40°C and returned back to the initial value under gradual cooling. Maximum current obtained from the thylakoid-based SC was 0.46 μA, while maximum current generated by the anthocyanin-based SC was 1.75 μA. The obtained results allow to reveal new possibilities for increasing the efficiency and stability of the SCs on the basis of biological material. In addition, the wide spectral range of the created set-up also makes it possible to study the properties of the SCs-based on chlorophylls absorbing low-energy photons of light, as well as the components of the photosynthetic apparatus comprising these forms of chlorophyll. Such SCs will be very promising for expanding the effective spectral range of the SCs.

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Lecture

Advances in terpene bioproduction in fast-growing cyanobacteria

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Climate change threatens global ecosystems, national securities, and human health. Cyanobacteria can help combat this because of their high photosynthetic efficiency and potential for carbon conversion into bioproducts, thus making carbon capture profitable.

Toward that goal, we are engineering cyanobacteria for production of isoprene (C_5H_8) and β-pinene ($C_{10}H_{16}$), which are precursors for thousands of terpenes including synthetic rubber, pharmaceuticals, and high-density fuels. Terpenes can be made via the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway whose products are isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). Cyanobacteria possess the MEP pathway but lack an isoprene synthase (IspS) enzyme for converting DMAPP to isoprene, and lack a mono-terpene synthase for converting geranyl diphosphate (GPP) to pinene. We have expressed synthetic, optimized *IspS* and IPP-DMAPP isomerase (*IDI*) genes in *Synechococcus* PCC 7002 cyanobacteria and produced isoprene at rates up to 80 times higher than first reported for cyanobacteria. Optimized GPP synthase and β-pinene synthase genes have also been expressed at high levels but β-pinene has not yet been detected, possibly because of toxicity, and modified genes are being tested. Isoprene production, which serves as a general proxy for terpene bioproduction, has been enhanced by temperature-regulated expression of potentially toxic MEP genes, added copies of *IspS-IDI*, MEP, and RuBisCO genes, together with inactivation of glycogen synthesis. Currently, our best strain produces isoprene at ~10 mg g DW^{-1} 12h day^{-1}, or ~5% of captured carbon converted to isoprene. *Synechococcus* strains have been maintained in a 100 liter greenhouse photobioreactor with continuous isoprene production under natural day-night cycles and fluctuating light intensities that are lethal to many microalgae. Isoprene production has also been obtained in ultra-fast-growing *Synechcococcus* UTEX 2973 and we are exploring several strategies for further enhancement, including immobilization. Our findings demonstrate stable isoprene production in fast-growing cyanobacteria, which are attractive platforms for bioproduction in photobioreactors that use wastewaters and flue gases from industries or biodigestors as nutrient and CO_2 sources. Two patents have been issued on the technology and the work was supported by the UW WiSys Technology Foundation, UW Center for Technology Commercialization, and US National Science Foundation STTR Phase I-IB grants.

Lecture

Metabolic engineering of microalgae as green cell factories for fuel production

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Photoautotrophic organisms are capable of efficiently converting inorganic CO_2 with sunlight energy and by water splitting into chemical energy and eventually into organic biomass. In particular microalgae have the potential to serve as sustainable biocatalysts for direct sun-to-bioproduct approaches, e.g. for the synthesis of biofuels. An efficient photon conversion efficiency of sunlight into biofuels of interest however requires the availability of genetic tools for the generation of mutants, which have the capability to serve as powerful green cell factories. Recent advanced metabolic engineering approaches to directly produce synthetic carbon-based liquid fuels as well as new strategies to improve bio-hydrogen production in microalgae will be presented. In addition, results from a systematic genetic and molecular characterization of the green miroalga *Botryococcus braunii* as a potential green cell factory for hydrocarbon production will be discussed.

Lecture

From natural to artificial photosynthesis

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The utilization of solar energy by oxygenic photosynthesis allowed cyanobacteria, algae and higher plants to conquer the world and changed life on Earth in a dramatic way. In my talk I first summarize the principles of oxygenic photosynthesis. On that basis, I present recent results on the mechanism of water oxidation, including the bicarbonate effect. Finally, I outline the principles of artificial photosynthesis and its potential for producing solar fuels.

Lecture

Carbon flow into lipids: A regulatory mechanism in seed oil biosynthesis in biofuel tree species

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Rising atmospheric CO_2 concentration and the depletion of fossil fuel stocks have created a demand for secure supplies of carbon-neutral substitute fuels. Petro-based oil meets about 95% of the requirement for transportation fuels, and the demand has been steadily rising. There is a resurgence of interest in recent years for alternate energy, including biomass-based first generation and oleaginous plant-based second generation biofuels. During the last ten years, certain non-edible oil seed trees like *Jatropha curcas, Pongamia pinnata, Simarouba glauca, Madhuca india and Calophyllum inophyllum* as well as certain microalgae have been advocated as promising and potential feed stocks for biofuel production with economically sustainable and environmental benefits. This talk analyses the state-of-the art understanding of the use of non-edible oil seeds as alternative/additional biofuel energy resources. The non-edible seeds of the above mentioned tree species contain approximately 35–40% oil by dry weight and the transesterification of these oils yield high quality biodiesel for its use as the best blend with petroleum fuel. Understanding the biosynthesis, modification and improvement of these non-edible oils is critical to make them more sustainable and economically viable biofuel resources. This presentation narrows down to elucidate the vibrant and dynamic nature of triacylglycerol metabolism during the seed ontogeny to provide certain crucial regulatory events in the metabolic network for quantitative and qualitative improvement of oil in the biofuel feed stock. Metabolic profiling of the inner integument has clearly shown significant accumulation of photosynthetically derived sucrose in the early stages of seed development, which was possibly utilized by the endosperm for its development at later stages. Further confirmation on this utilization of stored carbohydrates in the inner integument by the endosperm into total lipids was achieved through ^{14}C-labeled sucrose and glucose incorporation studies. Our data provide a direct evidence for the reprograming of fatty acyl fluxes during storage lipid synthesis, which is crucial for metabolic regulation in the qualitative improvement of seed oil as an efficient biofuel.

Lecture

Biology and engineering innovations to impact photosynthesis and algal productivity

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One of the significant focus areas for Reliance Industries is in the area of renewable energy using microalgae and to innovate and exploit the most important chemical reaction on earth, which is photosynthesis. This presentation will mainly cover the role of synthetic biology and engineering innovations to improve algae productivity including photosynthesis.

Innovations in biology, especially synthetic biology has made it easier to leverage living micro-organisms to produce products useful for human life and civilization. We at RIL have developed cutting edge tools and technologies for synthetic biology to utilize the fullest potential of this opportunity. We are exploring the use of micro-organisms like algae and natural photosynthesis, which forms the fundamental basis for bio-crude and other value added products such as proteins from algae. Algae, in particular, are highly efficient convertors of sunlight to stored energy. Advances in synthetic biology and gene editing can enable significant increases in productivity or overall photosynthesis.

Growth of algae is not limited to two dimension as in plant and this offers an advantage and opportunity to utilize engineering innovations in light management to impact photosynthesis directly and thereby improving productivity. Our efforts are also directed to understand kinetics of various steps in conversion of photons to stored energy in the biomass.

Coupled with the availability of different high-throughput technologies and bioinformatics platform along with innovative engineering breakthroughs, algae can potentially provide opportunities to significantly impact different facets of human life and civilization. This presentation will cover use of modern biology and engineering tools to improve algal photosynthesis.

Lecture

Modification of cyanobacteria for the produce useful compounds

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Microalgae attract attention as the cell factory to produce useful compounds via photosynthesis. Cultivation, synthesis of artificial compounds, cell harvesting and extraction of the product are the major energy consuming steps during the process. We attempted to improve the production by the development of a novel sensor to regulate gene expression, modification of fatty acid metabolism to produce novel fatty acids and autolysis system of the cells to release the products from the cells into the media.

To switch gene expression to modulate the metabolism by the artificial stimuli, we considered that water-insoluble gaseous compounds might be a good candidate. Because after stopping the exposure the signal is immediately removed from the medium. We can repetitively activate or inactivate during the culture and also recycle the medium. We developed a toluene-sensor which functions in the cyanobacterial cells.

The photosynthetic organisms synthesize saturated and (poly)unsaturated fatty acids. The unsaturated fatty acids are very sensitive to oxidation by O_2. We introduced genes for modification of the fatty acids to alter the oleic acid (18:1) in the membrane lipids into cyclopropane or methyl-brunched fatty acids. Production of the modified fatty acids did not severely affect the activity of photosynthesis, suggesting the possibility of production of modified fatty acids via photosynthesis.

Lastly, we expressed the cell-lysis enzymes under control of the *phoA* promoter induced by P_i-deficiency. Up to 80% of the cells were lysed under the P_i-deficient conditions. This system may assist recovery of the products accumulated intracellularly by the consumption of the P_i from the media.

Poster

Association of miRNA associated with chlorophyll biosynthesis and regeneration capacity in scutellum derived calli of rice

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Certain photosynthetic genes targeted by miR395, miR6251, miR393, miR444 in rice while in maize miR395, miR1423, miR528, miR399, miR171 are important in regulating photosynthesis [1]. Therefore, these miRNA can be important players in regulating the greening and regeneration in rice callus system. The rice scutellum derived calli for *in vitro* culture is dependent on the many factors which include different media compositions. Variable concentration of phytohormones shows differential regeneration ability, the appearance of green spot and emergence of plantlets which suggest the differences in photosynthesis mechanism during the process of in-vitro culture. Differentiation in a calli is identified by the green spots appearance which is the evidence of active photosynthetic machinery. Literature suggests higher expression miRNA osa-MIR169a,i,k*, h-m,q, osa-MIR164a-f, osa-MIR167d,f-h,j osa-MIR159a-2,b, osa-MIR444b-1,c-1, osa-MIR159a-1,c-e,f , osa-MIR171i, osa-MIR160a-d, osa-MIR156l, osa-MIR166i-j, osa-MIR394, osa-MIR5150-5p osa-MIR5156-5p osa-MIR5150-3p osa-MIR5158-3p osa-MIR5157a-b-5p osa-MIR1873, osa-MIR1861h*,h,j, osa-MIR1859, osa-MIR5157a-b-3p, osa-MIR827a-b in differentiated and undifferentiated calli [2]. These miRNAs target genes which are involved in leaf development, leaf polarity, auxin signaling. Recent addition to our knowledge of miRNA regulation in rice suggests 16.3% of miRNA are involved in photosynthesis [3]. Apart from the given miRNAs, certain miRNAs involved in leaf senescence such as miR172, miR159, miR167, miR171, Pre miR131, Pre miR11 regulate the genes such as AP2, MYB, LRR-RLKs, psbA. These genes are known to regulate the chloroplast development, chlorophyll accumulation, PSII activity thereby modulating photosynthesis [4]. We present herein psRNA target scan data for the targets associated with chlorophyll biosynthesis and plant development for the miRNA associated with plant regeneration capacity in *Oryza sativa*.

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Poster

Thylakoid model analysis of fluorescence and P700 redox transients in dark-adapted and preilluminated pea leaves

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The photoinduced P700 oxidation (ΔA_{810}) paralleling the chlorophyll *a* fluorescence transients were monitored in the time range from 50 μs to 30 s on pea (*Pisum sativum*) leaves adapted to darkness for 15 min prior to dark-light transitions. Under light action at PFD of 200 μmol photons m^{-2} s^{-1} the fluorescence induction (FI) and $P700^+$ redox changes (ΔA_{810}) display O(JI)PSMT and concurrently OABCD kinetic stages.

Transients of FI and ΔA_{810} in dark-adapted pea leaves were reproduced in their parallel and antiparallel phases within the framework of the Thylakoid-membrane (T-M) model fitted to both FI and ΔA_{810} data on the time scale extended from 20 s [1] up to 30 s. The previous T-M model [1] has been refined with the time-dependent rate constant to model $NADP^+$ reduction by ferredoxin supposing the FNR activation in the time domain of 20–40 s after the start of illumination. The dynamic modification of the rate constant of radiationless excitation energy dissipation in PSII antenna (k_D) was needed to fit the FI decline and $P700^+$ oxidation levels. We suggested that the high energy qE quenching is triggered by low lumenal pH. Then, the k_D increase from the basic value was described by a Hill-type equation with the Michaelis constant corresponding to a switch point at pH 5.4.

In addition, dark-adapted (15 min) leaves were preilluminated for 10 s, and then darkened for 10 s prior to 30-s illumination in order to obtain the FI and ΔA_{810} data. As a result, the induction OJ(I)PSMT and OABCD kinetics were strongly altered on the 30 s time scale. The present T-M model simulations revealed certain effects of preillumination. The fast OJ(I)P stages are simulated with an assumption that semiquinone fraction (with reduced Q_B^-) initially exists before the 30 s light period. The intermediary stage of $P700^+$ reduction and the second wave of P700 oxidation–reduction might be explained by the increased acidification of lumen. Further investigations are necessary to find out the mechanisms of ΔA_{810} and FI correlation on the 30 s time scale due to processes associated with and subsequent to 10 s preillumination.

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Poster

Quantifying fluorescence yield and O_2 generation in algal or cyanobacterial samples with Photosystem II and Thylakoid membrane models

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Comparative studies of alga/cyanobacteria afford a basis to analyze coordinated thylakoid membrane processes in oxygenic photosynthetic organisms. Research might be proposed for monitoring the bioefficiency of laboratory or natural phytoplankton system. Dark-to-light transients of Chl *a* fluorescence induction (FI) revealed clear-up different patterns for the alga/cyanobacteria strains [1]. Excitation trapping, the ensuing electron transport, transmembrane proton/ion transfer, filling pools of PQH_2, Fd^r, NADHP, ATP impact the FI kinetics. We developed kinetic models of photosystem II (PSII) [2], thylakoid membrane (T-M) [3] in order to analyze both the fast OJIP and slow PSMT FI stages.

The FI curves were measured by own designed custom-build fluorometers: stationary "MEGA 25" and a portable device [4]. PSII model fitting to FI data measured on laboratory *Chlorella* strain after 10 min dark adaptation provides the main parameters of electron transfer in reaction centers (RCs). Then distinct FI patterns were measured on phytoplankton samples from the cascade (r. Temernik, Rostov-on-Don) of ponds: upstream insanitary № 1, renovated № 2 with high biological productivity, lower № 3 with blooming water. Parameters similar to those for the laboratory *Chlorella* fit the FI data of control pond № 2. The fluorescence contribution of Chl not associated with the PSII is shown higher by 50% and 100% (ponds № 3 and 1) probably due to an increasing content of cyanobacteria. Calculations "pond № 1" show the 10-fold reduction effect in the generation of O_2 in RCs, that can be explained by inhibition of OEC as compared with phytoplankton of "ponds № 2, 3». For laboratory strains of alga/cyanobacteria the FI measurements in 5 min time interval are planned to be reproduced by the T-M model calculations.

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Poster

Cathodic and anodic photocurrents from *Rhodobacter sphaeroides* reaction centers immobilized on titanium dioxide

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One of the biggest problems of the contemporary world is the depletion of fossil fuels. Among possible solutions for meeting future energy demands, solar cells based on photosynthetic reaction centers (RCs) of the purple bacterium *Rhodobacter sphaeroides* are considered. The proposed construction is similar to that of Dye Sensitized Solar Cells invented by Michael Graetzel, but where natural pigment proteins are used instead of the artificial dye. For the described research new genetically engineered RCs were used in which a TiO_2-binding peptide tag was added. As an redox mediator TMPD (*N,N,N′,N′*-tetramethyl-*p*-phenylenediamine) was used. TiO_2 layer was prepared using pastes produced following two different procedures and using two different nanoparticle sizes. Furthermore some samples were treated with $TiCl_4$.

Depending on preparation procedure cathodic or anodic currents of the order of up to a few μA cm^{-2} were obtained. For explanation of the observed photocurrents, a mathematical kinetic model is proposed that includes: (1) an anodic current due to injection of electrons from the triplet state of the RC primary donor (P) to the TiO_2 conduction band, (2) a cathodic current due to reduction of P^+ by surface states of TiO_2, and (3) transient cathodic and anodic peaks due to oxidation/reduction of TMPD/$TMPD^+$ on the conductive glass (FTO) substrate.

Understanding of this mechanism will hopefully enable further optimization of our prototype solar cell.

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Poster

Integration of trimeric PS I stabilized within styrene maleic acid lipid particle into biohybrid solar device

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The influx of CO_2 and methane into the atmosphere via carbon fuel combustion is the underlying cause of global climate change. This influx exacerbates rising global temperatures, acidification of the oceans, and rising sea levels. Additionally, oil and coal are approaching peak levels, exemplifying the urgent need for new energy cultivation strategies to accommodate a growing global population. The natural process of photosynthesis offers a potential strategy; direct solar energy conversion. In plants, algae and cyanobacteria, chlorophyll (chl) and accessory pigments become excited upon illumination and pass their excitation energy to one of two specialized pigment protein complexes, called reaction centers. One such membrane bound reaction center, photosystem 1 (PSI), converts the excitation energy to a charge separated state, causing the photooxidation of the reaction center with a quantum efficiency (QE) approaching 100% [1]. Thus far, attempts to fabricate biohybrid solar devices (BHSD) using PSI, though robust, yield very low QE. Current extraction methods used to isolate membrane bound proteins replace native lipids with expensive surfactants, such as n-dodecyl-β-D-maltoside (DDM). However, it remains unclear if this approach alters the conformation, function, pigment or cofactor content. A new technique, utilizing styrene maleic acid (SMA) alternating copolymer has been used to isolate membrane bound proteins retaining their native boundary lipids [2]. In this study, trimeric PSI from the cyanobacteria *Thermosynechococcus elongatus* (Te) has been successfully stabilized within a styrene maleic acid lipid particle (SMALP). To date, this is the largest membrane bound protein complex to be isolated using this technique. The shape and structure of the PSI-SMALP has been examined using small angle neutron scattering (SANS) and small angle X-ray scattering (SAXS). The resulting PSI-SMALP has been biophysically characterized using low temperature fluorescence spectroscopy and dynamic light scattering. The rates of electron transfer and re-reduction of PSI in solution with native electron donor cytochrome c6 from Te has been analyzed using laser flash photolysis. This work presents a diffusion-free, BHSD composed of trimeric PSI-SMALP. The effects of maintaining the native lipid environment and carotenoids within the PSI-SMALP regarding QE of the device, or incident photon conversion to electron (IPCE), are also reported.

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Poster

Electropotential driven semi-artificial photosynthesis in biocatalyzed photoelectrochemical system

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Photosynthesis mechanism is a multistep process; it harvests the solar energy, transfers the excitation energy in the form electrons to redox equivalents like $NADP^+$, splits water molecule into H^+ and O_2, synthesizes ATP and carbohydrates from CO_2. Photosynthesis is known to be having limitations in terms of converting the incident quantum of light to excitation of the pigment molecules; antennae of chlorophyll molecules show maximum absorbance of light with the wavelength of ~450 nm (blue light). In environmental light fluctuating regions with very less or very high light intensity show scanty growth and reduced performance of photosynthetic activity. In this study we propose to overcome light dependent state of photosynthesis, whose cause can be attributed to the reversible changes occurring in the chlorophyll *a*/*b* molecules which are the primary antennae components of the light harvesting components (PS II and PS I). Biocatalyzed photoelectrochemical system (500 ml) was designed and used to study the experimental objective by applying potential which is the additional source of energy for excitation and/or water splitting. Different potentials (400, 600, 800 and 1000 mV) were applied by a potentiostat in both light and dark conditions to photosynthetic microalgae, cultivated under autotrophic mode of nutrition with CO_2 as sole substrate at pH 7 with controls (under light and dark conditions without any applied potential). Biomass, carbohydrate, lipids and chlorophyll *a*/*b* pigments were quantified and analyzed in both light and dark conditions along with pH monitoring during 22 days of operation. System operated at 400 mV showed maximum biomass growth (0.99 g/l) compared to control (0.65 g/l) operation while under dark conditions 600 mV showed maximum growth (0.49 g/l) compared to control (0.01 g/l). These observations suggest that applied potential to the photosynthetic cultures could overcome certain limitations of photosynthesis. As results show enhanced biomass growth (potential source for value added compounds like lipids, algal oil, etc.) and higher carbohydrate synthesis which also correlates to higher carbon dioxide sequestration advocating cleaner and greener environment by the novice application of BES.

Poster

Metabolic engineering of cyanobacteria for the photosynthetic production of sorbitol

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Photosynthetic production of valuable products form carbon dioxide can be leveraged to address problems of global warming and resources. Cyanobacteria, which are photoautotrophic prokaryotes, have been studied to apply biomaterial productions via photosynthesis. Sugar alcohols are attractive compounds for the photosynthetic production, since they are produced from sugars via the Calvin cycle and reducing power via the light reaction in phototrophs.

We focused on sorbitol as the target product in cyanobacteria. Genes of NAD-dependent and NADP-dependent sorbitol-6-phosohate dehydrogenase, *srlD2* and *s6pdh*, were introduced into a model cyanobacterium *Synechocystis* sp. PCC 6803, respectively. The strain constitutively expressing an *srlD2* gene grew normally but did not produce sorbitol. The introduction of an *s6pdh* gene with strong constitutive promoter was impossible, so that the *s6pdh* gene was expressed with theophylline-inducible riboswitch. This transformant successfully produced 11 mg/L of sorbitol in the culture with theophylline induction, whereas the sorbitol-producing cells died soon.

We postulated that the fatal growth inhibition was due to shortage of either carbon source or NADPH, and overexpressed two enzymes: fructose-1,6-bisphospahatase (FBPase), which is one of rate-limiting enzymes in Calvin cycle; and membrane-bound transhydrogenase (PntAB), which catalyzes the electron transfer directly from NADH to $NADP^+$ at membrane potential. The strains overexpressing these enzymes greatly alleviated the growth inhibition, and the viability of cells was more improved by FBPase. On the other hand, the sorbitol production rate was significantly accelerated by PntAB overexpression. As a result, the triple strain (S6PDH/FBPase/PntAB) achieved sustainable production of sorbitol (202 mg/L in the culture). We will discuss critical issues for metabolic engineering of photosynthetic production in cyanobacteria.

Poster

NADPH-fluorescence: an intrinsic probe for the characterization of recombinant oxidoreductases in cyanobacteria

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Oxidoreductases are highly selective enzymes that catalyze oxidative redox reactions under mild conditions. They are already established for the environmental friendly production of pharmaceuticals and fine chemicals. We have recently shown that cyanobacteria are promising hosts for the expression of recombinant oxidoreductases, which are coupled to photosynthetic electron transfer for light-driven biotransformations [1–3]. Here, we introduce NADPH-Fluorescence as a versatile probe for the characterization of recombinant NADPH-dependent oxidoreductases *in vivo*. Measurement of light-dark NADPH-fluorescence transients in the presence and absence of different substrates allows direct determination of specific rate constants that can be compared with *in vitro* data. Furthermore, inhibition of alternative electron sinks may allow streamlining of electrons towards product formation, which can be monitored by NADPH-fluorescence.

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Poster

Rice cultivars with varied tolerance towards high light and UV-B irradiation: A comparative physiochemical approach

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Solar irradiation influences photosynthesis and photomorphogenic responses in plants, but high intensity light and its integral ultraviolet (UV) part can induce stress responses in plants. In this study, the photochemical process in three cultivars of *Oryza sativa* L. (Aathira, Mangalamahsuri and Swarnaprabha) exposed to varying intensities of high light and UV-B radiation was investigated. Rice seedlings were exposed to high light stress of 2000 µmol m^{-2} s^{-1} (2, 4, 6 and 8 h) and UV-B (7, 14, 21 and 28 kJ m^{-2} d^{-1}), the chlorophyll and carotenoids content, photosystem (PS) I and PSII activity, chlorophyll *a* fluorescence transients, mitochondrial activity, enzymatic (superoxide dismutase and ascorbate peroxidase) and non enzymatic (total phenolics and ascorbate) antioxidants were measured. It was revealed that Aathira was the most tolerant cultivar towards both stresses and Swarnaprabha was the most sensitive cultivar in response to both high light and UV-B irradiation. Mangalamahsuri exhibited an interesting stress tolerance response, it was highly tolerant towards UV-B and at the same time highly susceptible towards high light; thus here it was inversely modulated in response to UV-B and high light stress. The inhibition of photochemical traits occurs as a result of more PSI and PSII damages as evidenced from the reduced photosynthetic O_2 evolution and cholorophyll *a* fluorescence parameters in the seedlings of Swarnaprabha, exposed to high light/UV-B. The photosynthetic performance of Aathira did not show major reduction under high light/UV-B exposure as compared to control seedlings. However, this cultivar exhibited high activities of both enzymatic and non enzymatic antioxidants and thus afforded more protection from reactive oxygen species induced by excess light and UV-B. The inversely modulated photosynthetic performance and upregulated level of antioxidants observed in Mangalamahsuri exposed to high light/UV-B deserves a special attention. Although high light and UV-B are integral parts of solar radiation, the response of the plants towards these stresses were varied and opposite, even in the same cultivar, indicating that the tolerance mechanism may be of entirely different nature in both these stresses.

Poster

Formation of gold nanoparticles by thylakoid membranes

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Gold nanoparticles have attracted much attention in many areas of physics, chemistry, and artificial photosynthesis because of their unique physicochemical properties compared to those of the bulk gold crystals. In order to fully utilize these unique properties in basic science, a necessary step is the ability to synthesize nanoparticles with high monodispersity. Several reports on microbial synthesis of gold dispersed nanoparticles have been published. However, the molecular mechanisms of such synthesis remains unclear. Kikuchi and co-workers reported that the formation of gold nanoparticle from *Lactobacillus casei*. They reported the galactolipids play important roles of reducing Au atoms. In this study, we performed to synthesis gold nanoparticles from thylakoid membranes. In general, neutral galactolipids monogalactosyldiacylglycerol and digalactosyldiacylglycerol are predominant (about 80% of total lipids) in thylakoid membranes in plant and cyanobacteria. When we mixed thylakoid membranes and $HAuCl_4$ solution, the colour of suspension turned from green-yellow to purple. The purple suspension had an absorption maximum at 540 nm. The unique feature of gold nanoparticles is their optical properties due to surface plasmon resonance (SPR).

We will discuss the formation mechanism and applications of gold nanoparticles for artificial photosynthesis.

Poster

Sustainability of thylakoids-sensitized solar cell based on TiO_2

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One of the viable alternatives to high-cost silicon photoelements is the solar cell (SC) based on photosynthetic pigment-protein complexes, deposited onto the conductive substrate. These materials are biodegradable and renewable, abundant and low-cost. Also, the internal quantum efficiency of the charge separation step in natural photosynthesis is almost 100%. Photosystems I (PSI) and II (PSII) of plant or cyanobacteria, whole thylakoid membrane and bacterial reaction centers are used as the sensitizer in different works. Using mesoporous TiO_2 substrate is a promising method, owing to its biocompatibility, corrosion resistance and large effective surface area.

Thylakoid membranes with all electron transport chain (ETC) components have advantages below: their extraction is easier than for single photosystems; whole ETC does not need special electron donor (water serve as one). Disadvantages of these SCs are following: small adsorption cross section; the narrow absorption band; low stability. Mesoporous substrate and external antenna complexes addition can help to solve first two problems.

In our laboratory was shown that thylakoid-sensitized SC with TiO_2 does not sustainable to the long-term storage and to the high temperature.

Under the temperature about 50°C, the current falls by about 95% relative to its maximum value at 22°C. For 20 days storage at room temperature, photocurrent fell by almost 100% (temperature of measurement about 20°C). Low stability of thylakoidbased SC relates with high-susceptibility of biological membrane and is an important problem for these SCs. It can be increased by adding of stabilizer molecules to the membrane preparation before its deposition.

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Poster

Impact of *Bacillus subtilis* (BS) treatment on the photochemical and antioxidant mechanism in okra exposed to drought stress and recovery

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Drought is an important stress factor influencing plant growth and yield. Some soil bacteria are known to improve plant growth under drought stress. The aim of the present investigation was to find out the changes involved in the photosynthetic and antioxidant mechanisms in okra (*Abelmoschus esculentus* (L.) Moench) plants treated with *Bacillus subtilis* (BS). The BS treated plants were exposed to drought and re watering conditions for evaluating the means of chlorophyll *a, b* stability index, carotenoids content, relative water content (RWC), osmolyte content, PSI (photosystem I), PSII (photosystem II) activities and antioxidant enzymes activity. BS treated plants ameliorate drought induced reduction in stability of chlorophyll *a, b*, RWC and PSI, PSII activity. BS treated plants also exhibited the increased accumulation of osmolytes such as sugar and proline under drought stress. Enhanced activity of enzymatic anti oxidants like SOD (superoxide dismutase), APX (ascorbate peroxidase) and CAT (catalase) were also observed in BS treated plants under drought stress. Upon re-watering, BS treated plants quickly recovered chlorophyll *a*, *b* stability, RWC, and PSI, PSII activity compared to BS untreated control plants. Reduction in the antioxidant enzymes and osmolytes accumulation is also observed during re-watering. The augmentation of antioxidant enzyme activity and photosynthetic yield by BS treatment in drought stressed plants alleviates the harmful effect of drought in okra and can be suggested as a rewarding means in overcoming drought.

Poster

Trophic optimization of *Chlorella sorokiniana* SVMBIOEN2 towards enhancement of neutral lipids (TAG)

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Microalgae are photosynthetic biomachines fostering humanity and sustaining life in multiple dimensions of biofuels, food, pharmaceuticals, nutraceutical and healthcare sector. Photobiotechnology platform is gaining grounds in biocapture of solar energy and CO_2 towards transformation into high value bio-based products. The photosystem bioarchitecture is arranged in arrays for harnessing energy from photons and convert into valuable metabolites. These photostructures present in the biological inert matrices of microalgal chloroplasts are treasure box of biotechnological applications. Microalgae have adapted to grow in various nutritional environments like autotrophic, mixotrophic and heterotrophic systems due to their metabolic versatility and resilience. Mixotrophic and heterotrophic cultivations are key technologies for achieving high biomass, lipid productivities and scaling up of algal bioprocess. Optimization of stress conditions for lipid induction and fatty acids is the need of the hour for microalgal cultivation. The isolated strain *Chlorella sorokiniana* SVMBIOEN2 was evaluated with design of experiments (DOE) methodology using Taguchi orthogonal array (OA) towards high neutral lipid/TAG producing conditions. Various triggering factors for synthesis of C16:0 and C18:0 fatty acids which are precursors for long chain fatty acids were studied to understand multi-parametric stressors and gain insights on underlying fatty acid (FA) synthesis mechanism. The fatty acids thus generated can be useful for biodiesel, edible oil and nutraceutical applications. Enhanced neutral lipid (TAG) productivities with targeted fatty acid profiles will pave new avenues for capturing spectrum of bioproducts in sustainable pathways.

Poster

Up-scaling of optimized biomass and lipid conditions in flat panel photobioreactor: Comparative evaluation of *Chlorella vulgaris* and mixed microalgae

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Mixed microalgae and algal strain *Chlorella vulgaris* was evaluated for higher biomass and lipid production in a flat panel photobioreactor (FPPBR) fabricated with 8 mm thick transparence glass with total/working volume 60/50 l. FPPBR was operated with optimized parameters for biomass (salinity, 2 g/l; pH 10.0; glucose, 5 g/l; $FeCl_3$, 0.5 mg/l) and lipid production (salinity, 1 g/l; pH 6.0; glucose, 5 g/l; $FeCl_3$, 0.5 mg/l) derived from DOE methodology. The FPPBR were operated at ambient light (180–240 microeinsteins per second per square meter) and temperature (25–29°C). During the operation process parameters like pH, temperature, growth rate, photosynthetic efficiency, chlorophyll concentration, quantum yield lipid production and FAME were analyzed to monitor the carbon conversion towards biomass and lipid productivity. Microalgae (consortia) showed maximum biomass (3.85 g/l) and lipid production (19.55%) followed by *Chlorella vulgaris* (2.1 g/l; 15.85%) with optimized biomass and lipid conditions. The FAME composition enumerated the high fraction of C16:0 and C18:0.

Poster

Photosystem I deposited on the conducting surface – origin of the excitation decay acceleration

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Photosystem I (PSI) is a well-described pigment-protein complex with a quantum efficiency of photon to electron conversion near unity. This promising feature combined with high stability beyond the natural lipid membrane causes this complex to become the object of interest for biophotovoltaic applications. The main evidence of intactness of the structure and function of PSI in many artificial systems was its photoelectrochemical response. However, an additional evidence of stability could be obtained by a detailed study of the first steps of energy and electron transfer processes. Time-resolved fluorescence studies of the excitation dynamics in PSI suspended in solution and PSI deposited onto FTO conductive glass as a substrate showed significant acceleration of the excitation decay in PSI after immobilization [1].

The main goal of this contribution is to focus on the possible origin of such acceleration. We formulated two alternative working hypotheses: 1) the acceleration results from electron injection from PSI to the conducting surface; 2) the acceleration is caused by dehydration and/or crowding of PSI proteins deposited on the glass substrate. To resolve this issue, femtosecond transient absorption experiments were performed, with PSI being prepared in three states: (1) in aqueous solution, (2) deposited and dried on glass surface (either conducting or non-conducting), and (3) deposited on glass (conducting) surface, but being in contact with aqueous solvent. The kinetic traces for all systems with PSI deposited on substrates are almost identical and they decay significantly faster than the kinetic traces of PSI in solution. Therefore, we conclude that the accelerated excitation decay in PSI-substrate systems is caused mostly by dense packing of proteins.

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Poster

Reduced graphene oxide as an electron mediator in photosystem I and II

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Photosynthesis converts light energy into the chemical energy. The quantum yield of photosynthetic energy and electron transfer is nearly 100% by the forces of natural selections. Exploiting this photovoltaic abilities of photosystem (PS) for biohybrid device is one of the key research themes for sustainable energy. Carbon materials are also potential candidate as a next-generation device. Among them, graphene is a new material for its remarkable electronic properties and strength. Graphene is an atomically thin layer of sp^2 hybridized carbon atoms arranged in a honeycomb lattice and most emphasized by physicists for its great carrier mobility. However, graphene is insoluble in common solvents. Therefore, we used graphene oxide (GO) and reduced graphene oxide (rGO) in this study. Because they are soluble in water. GO loses their electrical conducting property compared to graphene. However, photoreduction of GO recovers its electrochemical property.

When GO was used as an electron acceptor, the oxygen evolving activity was recovered in isolated PS II. This implies the electron was transferred from PS II to GO. To confirm the GO was reduced by PS II, we performed X-ray photoelectron spectroscopy (XPS). XPS analysis clearly showed the reduction of C=O bond of GO by photoinduced PS II. We also monitored the photoluminescence (PL) of GO in the infrared region, which monitors the redox state of carbon materials. PL also showed the reduction of GO by PS II. When GO was used as an electron donor, the oxygen consumption was increased in isolated PS I (Mehler reaction). Therefore, we clarified that the GO worked as a good electron mediator for PS I and II.

Poster

UV-B priming imparts NaCl and PEG stress tolerance potential to rice seedlings

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UV-B radiation constitutes a major abiotic stress factor, which negatively affect growth and productivity of crop plants including rice. However, on the other hand lower doses of UV-B radiation have a priming effect on crops. Plants produced from UV-B primed seeds shows enhanced growth, improved physiological features and also accelerates the stress tolerance potential in plants. In the present study, seeds of *Oryza sativa* L. cv. Kanchana were primed with UV-B radiation (6 kJ m^{-2} d^{-1}) and were further subjected to NaCl and PEG stress. The effect of UV-B priming in imparting NaCl and PEG stress tolerance to rice seedlings were analysed using various photosynthetic features and ROS (Reactive Oxygen Species) scavenging parameters. Total chlorophyll and carotenoids content as well as photosystem I and photosystem II activities were found to be significantly higher in the UV primed seedlings under controlled condition. When stress (NaCl and PEG) was imposed, high level of photosynthetic pigment contents, photosystem (I and II) activities, compatible solutes (sugar and proline) accumulation as well as enzymatic (superoxide dismutase (SOD), catalase, ascorbate peroxidase (APX)) and non-enzymatic (ascorbate and phenol) antioxidants was recorded in PN (UV-B Primed + NaCl stress) followed by PP (Primed + PEG stress), as compared to non-primed ones. The above results indicate that, UV-B priming in rice seedlings effectively enhances the NaCl stress tolerance potential in the rice to a greater extend as compared to PEG stress tolerance potential. The effective alleviation of stress through UV-B priming was highly prominent in rice seedlings subjected to NaCl stress.

Poster

Glycine-betaine allows more photocurrent generation in thylakoid-sensitized solar cells at the elevated temperature

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Development of the solar cells (SCs) based on the components of the photosynthetic apparatus is a promising area of alternative energy research. The low cost and environmentally friendly production and use are the advantages of these SCs.

One of the SCs problems is their short lifespan due to rapid protein denaturation. SCs decompose faster during an operation at elevated temperature. Glycine-betaine (GB) is zwitterionic water-soluble compound that acts as compatible solute in plant and bacterial cells. It participates in osmotic adjustment and protection of macromolecules. Stabilizing effect of exogenous GB on the extracted photosystems is known. Commercialized glycine-betaine is not very expensive. We assumed, that it would be good protectant of photosynthetic macromolecules immobilized onto electrode in the SC. In our experiments, a photocurrent of TiO_2-based thylakoid-sensitized SCs was measured at different values of the ambient temperature. We compared the SCs with and without GB that was added into the sensitizer mixture before it was deposited onto the TiO_2 surface. At temperatures approaching 50°C, the photocurrent of SC without GB falls by approximately 95% relative to its maximum value. But for GB-containing SC, the photocurrent decreased by about only 38% relative to its maximum value.

It has been shown that GB can directly stabilize the Photosystem II complex against dissociation of the extrinsic proteins of the oxygen evolving complex. It was also suggested that GB might behave as a solute that is excluded from the charged surface domains of proteins, and thereby permit better access to redox active molecules. These activities may be more prominent at higher temperature and over extended time periods.

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Section 1.9: Regulation of Photosynthesis and Environmental Stress

Lecture

Photosynthesis and nitrogen fixation (Photodiazotrophy) under stress: cyanobacterial remedies

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As naturally abundant, photo-diazotrophic, heterocystous cyanobacteria, *Anabaena* spp. abound in tropical soils and waters, and contribute significantly to the carbon and nitrogen economy of soils. Heterocystous cyanobacteria have the unique distinction of being the only microbes capable of carrying out the oxygen-evolving photosynthesis and highly oxygen-sensitive nitrogen fixation simultaneously in light. Both the vital processes of photosynthesis and nitrogen fixation are highly interdependent in *Anabaena* and very sensitive to environmental stresses. Our studies on cyanobacterial response to nutrient deficiency (N, P, K), salinity and osmotic stress, heat-shock, heavy metals (uranium), pesticides (lindane), ionizing radiations (γ-rays) and other stresses have identified oxidative stress generation as a common denominator underlying all kinds of stresses, and revealed the impairment of specific cellular targets during stress.

Oxidative stress management is inherent to the oxygenic life style of *Anabaena* spp. which are endowed with two superoxide dismutases, two catalases and 7 peroxidases, in addition to several non-enzymatic mechanisms of ROS alleviation. However, their basal or stress-induced levels are often not adequate to cope with the magnitude of stress. We have developed new tools for genetic transformation and solar-powered gene expression in *Anabaena*. This has led to cloning, characterization and over-expression of many candidate genes to enable survival and enhance photosynthesis and nitrogen fixation capabilities of *Anabaena* in stressful environments.

Lecture

Regulation of photosynthetic light reactions in the thylakoid membrane – an evolutionary view

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Linear electron transfer chain of oxygenic photosynthetic organisms is rather similar from cyanobacteria to higher plants. On the contrary, the light harvesting systems and various regulation mechanisms of energy distribution/dissipation and electron transfer pathways show distinct evolution from cyanobacteria to algae, mosses, conifers and finally the angiosperms. Development of chlorophyll *b*-containing light harvesting systems and complex regulatory networks of energy and electron transfer reactions led to the development of distinct lateral heterogeneity of the thylakoid membrane. Light-induced dynamics in lateral heterogeneity of higher plant thylakoid membrane allows fluent photosynthetic electron transfer and equal light harvesting capacity as well as efficient photoprotection of both photosystems in response to changes in the light environment. On the contrary, the fluency of electron flow in cyanobacteria thylakoid membrane is largely dependent on a broad range of electron valves that have gradually disappeared during evolution of plant chloroplasts. After a demonstration of the lateral heterogeneity of the thylakoid membrane in higher plant chloroplasts in 1980, our knowledge on light-induced dynamics of such a heterogeneity has slowly evolved in parallel with increasing knowledge on the regulation of photosynthetic light reactions according to environmental and metabolic cues.

Lecture

Consequences of disturbance in chloroplast or mitochondrial redox in leaf discs of pea, *Pisum sativum*

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Reactive oxygen species (ROS) are produced as byproducts during various metabolic pathways that are localized in different cellular compartments, wherein the processes of photosynthesis and respiration take place. Oxidative stress occurs when ROS are not scavenged quickly and the rate of damage exceeds rate of repair. We have examined the pattern of ROS accumulation and its impact on metabolism in leaf discs of *Pisum sativum.* We have employed oxidants, which induce ROS in different compartments: acifluorfen methyl ester (AFM) and paraquat (PQ) (both in chloroplasts), and menadione (MD, in mitochondria). Responses were measured after 4 h in dark, moderate or high light. The accumulation of H_2O_2, superoxide and non-enzymatic antioxidants levels were measured. We have also monitored photosynthesis, respiration and changes in chloroplast pigments. An increase in enzymatic and non-enzymatic antioxidants, and decrease in rates of photosynthesis and respiration, was noticed on exposure to three oxidants, particularly in high light. These changes were stronger in PQ and AFM treated samples than MD treated. Total chlorophyll and carotenoid contents decreased, the decrease in carotenoids being stronger than that in chlorophylls. Our study indicates that changes in antioxidants and physiological activities seen in chloroplast targeted stress were stronger than those in mitochondria. Interestingly, photosynthesis was affected even during mitochondria targeted stress, indicating that oxidative stress, created in mitochondria or chloroplasts, affects both photosynthesis and respiration. Our results demonstrate that ROS generated in one compartment can also affect the metabolism in other compartments. Proline is often considered as an indicator of oxidative stress in plant cells. Besides the antioxidants of ascorbate and glutathione, the levels of proline, a compatible solute, increased. We propose that oxidative stress in chloroplasts promotes proline biosynthesis, while decreasing proline oxidation.

Lecture

Cytokinin mitigates Cd induced damage in growth and PS II photochemistry of *Trigonella foenum-graecum* L. seedlings by up-regulating the ascorbate-glutathione cycle

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In recent years, chlorophyll (Chl) *a* fluorescence analysis is considered as a highly sensitive, non-destructive, and reliable tool for measuring photosynthetic efficiency, particularly the photochemistry of photosystem II (PS II) under variable environmental conditions [1]. Cytokinin plays an important role in plant adaptation to environmental stresses [2]. Thus, in this study the effects of kinetin (KN; 10, 50 and 100 μM), a synthetic cytokinin, on the growth, photosynthesis, PS II photochemistry and ascorbate-glutathione cycle in *Trigonella foenum-graecum* L. seedlings were investigated under cadmium stress. Cadmium (Cd) at tested doses (3 mg Cd kg^{-1} soil and 9 mg Cd kg^{-1} soil) reduced the growth, pigment contents, photosynthetic O_2 evolution rate and carbonic anhydrase activity which were accompanied with an increase in H_2O_2 formation as a result of Cd accumulation in tissues. Further, to quantify the performance of photosystem (PS) II, chlorophyll *a* fluorescence (JIP test) was analyzed and under Cd stress the yield for primary photochemistry (φP_0), yield of electron transport per trapped exciton (ψ_0), quantum yield of electron transport (φE_0) and performance index of PS II (PI_{ABS}) were decreased while the values related to energy flux parameters were found to increase. Foliar application of kinetin at 10 and 50 μM significantly alleviated Cd induced toxicity on growth, PS II photochemistry and oxidative damage while with 100 μM KN the toxicity was further exacerbated. Importantly, KN at 10 and 50 μM doses enhanced the redox states of AsA and GSH, and the related enzyme activities involved in the AsA–GSH cycle such as ascorbate peroxidase, glutathione reductase and dehydroascorbate reductase in *Trigonella* grown under Cd stress. Present study concludes that exogenous kinetin treatment caused differential effects against the Cd toxicity showing an alleviating effect on growth and PS II photochemistry through maintaining the redox status (> ratios: AsA/DHA and GSH/GSSG) of cell by regulation of AsA-GSH cycle of *Trigonella foenum-graecum* L. seedlings at 10 and 50 μM KN while at 100 μM KN the down-regulation of AsA-GSH cycle did not support the growth and PS II activity of the test seedlings.

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Lecture

Loss in photosynthesis reprograms cellular metabolism to sustain sugar homeostasis in *Arabidopsis thaliana* during senescence and stress response: Induction of cell wall hydrolases

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Loss of photosynthesis during leaf senescence results in cellular sugar starvation in leaves. Since execution of senescence program is energy dependent, the cells collect respiratory sugars from other sources through metabolic reprogramming to sustain energy homeostasis. Although the nature of the metabolic reprogramming is not fully understood, we have demonstrated the loss of photosynthetic production of sugar as a signal in modulating and reprogramming metabolic network during senescence and stress response in *Arabidopsis thaliana*. The cell wall polysaccharides are the richest source of organic carbon in plants and the polysaccharides are known to be broken down by several wall bound hydrolases and are subsequently converted to respiratory sugars. A possible link between loss of photosynthesis and induction of cell wall hydrolases namely β-galactosidase, β-glucanase and β-glucosidase is suggested during leaf senescence and in senescing leaves experiencing abiotic stress. In *Arabidopsis thaliana* the loss in photosynthesis is accompanied by up-regulation of genes coding for the hydrolases and enhanced activity of these enzymes [1, 2]. Importantly, when senescing leaves experience abiotic stress like drought and nitrogen deficiency, senescence induced loss in photosynthesis is aggravated that results in further increase in the activity of these enzymes. Recovery of photosynthesis with concomitant suppression of enzyme activity on withdrawal of stress is suggestive of the photosynthetic modulation of the enzyme activity. Expression of genes coding for hydrolases and enhanced activity of the enzymes late during senescence support the proposition that the activity of the hydrolases in the catabolic network for the polysaccharide degradation is the terminal event of the senescence program and the wall polysaccharides are the last source of the respiratory sugars in providing energy to execute and complete the senescence program. Our data suggest the possible role of flavonoids and anthocyanins for the sustenance of cellular viability for the activity of cell wall hydrolases at terminal phase of senescence and during stress response when loss of photosynthesis is almost complete.

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Lecture

Plant acclimation to environmental light conditions: role of STN7 kinase

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Changes in the environmental light conditions lead to alterations in organization of the pigment-protein assemblies in chloroplasts due to occurrence of various acclimation pathways. The present study is focused on short-term (state transitions, the migration of external photosystem II antenna between photosystem II and photosystem I) and long-term (modulation of the photosystem II antenna size due to downregulation of the antenna proteins biosynthesis) adjustments in photosynthetic apparatus.

Arabidopsis STN7 knockout plants and wild type (WT) plants were transferred from low light (LL) to high light (HL) for 4 days of continuous illumination. The experimental evidence revealed that the ability of WT plants to perform state transitions was restricted during the first day after transferring to HL. However, the potential renewal of state transitions occurrence was observed after the third day in HL. State transitions occurrence in WT plants inversely correlated with the hydrogen peroxide level: this level increased after one day in HL and then decreased again after the third day, when WT plants succeeded to modulate, *i.e.* to reduce, the photosystem II antenna size and, therefore, to acclimate to new light conditions. However, such HL treatment led to the destroying of the photosynthetic machinery of STN7 knockout plants, demonstrating that the mutant plants were not able to perform long-term acclimation.

Previously we have shown that the reduction of the photosystem II antenna size was hampered in HL in barley leaves characterized by the high reduction level of the plastoquinone pool but the low hydrogen peroxide content. These conditions were achieved in HL by the the incubation of barley leaves in the medium contained catalase. Incubation of barley leaves in the medium contained hydrogen peroxide allowed reaching in LL the hydrogen peroxide level similar to that observed in leaves in HL, while the reduction level of the plastoquinone pool remained low. Such conditions led to the reduction of the photosystem II antenna size in LL, which was comparable to that observed under HL conditions.

It has been suggested that the elevated amount of hydrogen peroxide during the first day after transferring of plants to HL influences the activity or redox state of STN7 kinase, allowing the long-term acclimation to HL to be performed.

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Lecture

Unravelling the photosynthesis efficiency and lipid biosynthesis enzymes of *Chlamydomonas reinhardtii* under iron deprivation

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Iron (Fe) is an essential nutrient element for photosynthesis, both in higher plants and in green algae. Fe is a constituent of many proteins that participate in electron transport reactions. In our study, *Chlamydomonas reinhardtii* cells were grown in iron deficient conditions which were further used to study photosynthesis efficiency, physiological parameters, as well as the expression patterns of lipid biosynthesis proteins. Under this condition both photosystem I and II were dramatically reduced. Our results showed that Fe deficiency leads to formation of lipid droplets and accumulation of Triacylglycerol (TAGs). This occurs significantly between 24 and 48 hrs of iron-starvation. Accumulation of TAG's in these conditions were studied using fluorescence-activated cell sorting and thin layer chromatography analysis. Fatty Acids were also identified and quantified by gas chromatography mass spectrometry (GC-MS) as their methyl esters. The GC-MS results indicate that the accumulation of total fatty acid content is marginal during 0–72 hrs of growth conditions. The FT-IR spectroscopy suggests that substantial metabolic changes and closely related functional groups are differentiated under Fe deficient conditions. Interestingly, the TAG biosynthesis proteins DGAT2A and PDAT1 were increased under these conditions. This study provided us a number of targeted genes/enzymes can be used for a systematic metabolic engineering to produce high levels of lipid that may be suitable for conversion to liquid fuels.

Lecture

Singlet oxygen stress induces arrest of cellular translation

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Singlet oxygen ($^1O_2$) production in plant cells is associated with chloroplast activity under high light or in the dark under stress [1]. We have shown that singlet oxygen induces different types of programmed cell death dependent on its source [2]. Singlet oxygen is known to readily oxidize guanylate ribonucleotides (G) in nucleic acids. Indeed, oxidized guanine is a diagnostic tool for many mammalian diseases but its consequence is poorly understood in plants. Singlet oxygen reacts with RNA to form 8-hydroxyguanosine (8-oxoG). To further understand the ramification of $^1O_2$ bursts on cellular health we employed an efficient photodynamic source of $^1O_2$, rose bengal. $^1O_2$ induced a significant increase in the ratio of 8-oxoG to non-oxidized G without causing change in the redox state of the cytoplasm or cell organelles [3]. Similarly, H_2O_2 and dark-light transitions of the *flu* mutant were shown to increase 8-oxoG to G ratio *in-planta*. Interestingly, $^1O_2$, and H_2O_2, induced a class of transcripts that is related to transcripts stimulated by the application of 80S ribosome translation inhibitors, cycloheximide and homoharringtonine. The results imply that these transcripts could be induced by a common cause, i.e. translation-arrest of ribosomes. Indeed, increasing ratio of 8-oxoG to G can be shown to affect a direct decrease in the general translatability of cellular mRNA. The decrease in translation accelerated the turnover of labile protein repressors and that in-turn stimulated transcription from the genes that they control. The results present a scenario whereby $^1O_2$, and H_2O_2 can cause significant oxidation of cellular mRNA, decreasing mRNA translatability to the extent where it releases genes from the repression of short half-life repressors. The work provides a coherent basis for understanding why transcriptomes of diverse reactive oxygen species and environmental stresses show commonalties.

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Lecture

Glucose induces photosynthetic damage leading to viable but non-culturable (VBNC) state in *Rubrivivax benzoatilyticus* JA2^T

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Rubrivivax benzoatilyticus JA2^T is a phototrophic bacterium capable of utilising a wide variety of organics as carbon sourceIIndia. On the basis of 16S rRNA gene sequence analysis, strain JA2^T. However, when strain JA2 was attempted to grow on glucose as sole carbon source, the organism resulted in temporal reduction in cell size, loss in pigments, photosystems and ubiquinone. This led the organism to transit to a state of non-cultivability commonly described as viable but non-cultivable (VBNC) state. VBNC state is considered as a survival strategy adopted by few Gram-stain-negative bacteria in response to unfavourable environmental condition. It refers to a state of bacteria where they are in a state of low metabolic activity [2]. VBNC bacteria are viable but fail to grow on routine bacteriological media in the laboratory conditions and become culturable again once resuscitated [3].

Viability of strain JA2 was evidenced from positive mRNA (signal), confocal microscopy and flow cytometry. A tenfold increase in the saturated: unsaturated fatty acid content of the membrane indicates membrane rigidity. Proteome profiling of glucose grown cells of strain JA2 by isobaric tags for relative and absolute quantification (iTRAQ) indicated reduced energy demand, metabolism of carbohydrates, amino acids and shutting down entire photosynthesizing machinery. However, transcription and translation related proteins ere differentially regulated. The cascade of cellular events taking place in the VBNC state is discussed.

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Lecture

LHCSR3 impairs photosynthetic membrane complex assembly of *Chlamydomonas reinhardtii* under drought stress

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In green algae, *C. reinhardtii* LHCSR3 is expressed under high light stress to protect the photosynthetic apparatus from photodamage. Gene expression and proteomic studies indicate LHCSR3 expression under nutrient deficiency in ambient light. Major source for the ΔpH across the thylakoid membrane is electron transport chain which is hampered under nutrient stress, which in turn signal the expression of LHCSR3. It is not clear how this protein is expressed and its importance under nutrient deficient and low light condition. Here in our study we induced nutrient stress by using poly ethylene glycol that can mimic drought conditions. By using biochemical and biophysical approaches like lpBN-PAGE, PAM, and Circular-dichroism, we studied physiological, morphological, structural and functional changes in photosynthetic apparatus in *C. reinhadrtii*. Significant findings of this work are (i) Under drought, there is negligible damage to protein content of photosystems, but there is significant decrease in electron transport rate (ETR) and photosynthetic yield. (ii) There are intact core complexes in both photosystems, but assembly of core with peripheral antenna to form functional super/mega complexes of PSII is affected. (iii) Significant expression of LHCSR3 in drought stress indicates role of this protein in disassembly of photosynthetic complexes. (iv) Development of ΔpH across thylakoid membrane in drought stressed cells inspite of reduced electron transport chain can be due to decrease in levels of functional ATP synthase. Hence, we propose that alteration in assembly of photosynthetic membrane complexes under drought is one of the reason for decreased photosynthetic yield. Expression of LHCSR3 coupled with impaired assembly of PSII super and mega complexes under drought indicates the photo-protective adaptation under high light conditions.

Lecture

Identification and characterization of a novel chloroplast protein controlling non-photochemical quenching under fluctuating light

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Plants have mechanisms to acclimate to high-light conditions by extinction of the excess light energy. These mechanisms are important for plants to efficiently perform photosynthesis, and have to be controlled accurately. Non-photochemical quenching (NPQ) in the chloroplast thylakoid membrane is one such defense and has been well studied. NPQ is induced when light energy is in excess, and it dissipates this excess energy. The NPQ-related compounds include the carotenoids, e.g., zeaxanthin. When NPQ is induced, zeaxanthin accumulates in thylakoid membranes, associates with light-harvesting complexes (LHCs), and then dissipates the excess light energy from the LHCs as heat. Under low-intensity light, zeaxanthin is converted into violaxanthin by zeaxanthin epoxidase. Violaxanthin cannot dissipate energy as heat efficiently. Under intense light, violaxanthin is converted into zeaxanthin by violaxanthin de-epoxidase (VDE); a mutation in *NPQ1* that encodes this enzyme induces a phenotype with decreased qE activity. VDE activity is enhanced by acidification of the thylakoid lumen, which is accelerated by photosynthetic electron transport. A mutation in *NPQ4* that encodes the thylakoid membrane protein PsbS, abnormally decreases qE, indicating that PsbS contributes to qE induction. PsbS-dependent qE induction is also regulated by ΔpH across the thylakoid membrane. These mechanisms can dissipate absorbed light energy, and thus the control of NPQ depends on the environmental light intensity. Because sunlight intensity can fluctuate greatly, regulation of NPQ induction should impact photosynthetic performance. Although NPQ induction mechanisms are well understood, how NPQ is regulated in response to fluctuations in light is not.

We recently reported a novel gene named *FLAP1* (*Fluctuating-Light Acclimation Protein1*), that is involved in the high-light and fluctuating-light acclimation response in plants [1]. We demonstrated that LAP1 protein involved in inducing of NPQ. However, a relationship between LAP1 protein and NPQ has not been clarified. To clarify the relationship, we isolated double mutants lacking both LAP1 and NPQ4 or PGR5. From analysis of these mutants, physiological function of LAP1 and its relation with NPQ will be discussed.

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Lecture

Source sink relationship: can obnoxious gaseous pollutants such as Nitric Oxide become a source for enhancing photosynthetic productivity in marginal land – a hypothesis

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Nitric oxide (NO) is an obnoxious gaseous pollutant, which is also synthesized in living systems and acts as a signaling molecule at very low concentrations. Our studies on the effect of exogenous NO donor sodium nitroprusside (SNP) on pea thylakoid membranes *in vitro,* reveals its direct effect on the photosynthetic oxygen evolution and the chlorophyll fluorescence. The SNP-donated NO stimulates photosystem II electron transport rate and diminishes the evolution of molecular oxygen. Studies *in vivo* showed that net photosynthesis increased with reduced transpiration rate and stomatal conductance, leading to an increase in water use efficiency by SNP. Based on these results, we put forth the hypothesis that lower efficiency of water oxidation (measured by the photosynthetic oxygen evolution) combined with the enhancement in photosynthetic electron transport can be explored for utilizing this obnoxious gas as an exogenous source for enhancing photosynthesis in a water limited condition in marginal lands.

Lecture

Toxic effects of mercury on primary processes of photosynthesis in the cyanobacterium *Spirulina platensis*

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Excess use of pesticides, fungicides and extensive industrialization leads to the accumulation of toxic metals like mercury in aquatic environment. In this environment, this metal primarily affects the algal productivity by acting at the level of basic physiological process i.e., photosynthesis. Therefore in this investigation the effect of mercury (6-30 μM) was studied on photosynthetic electron transport as well as energy transfer on the economically important aquatic cyanobacterium, *Spirulina platensis* by incubating for 5 min in the presence of metal ion. Electron transport studies clearly demonstrated that photosystem II is more susceptible to low doses of mercury (6 μM) than that of photosystem I. Spectral measurements clearly indicated that the absorption capacity of phycocyanin is decreased when compared to other pigment proteins. In addition the energy transfer from phycocyanin to chlorophyll *a* in photosystem II gets altered due to toxicity of mercury. Both *in vivo* and *in vitro* studies proved that mercury is binding to the β-subunit of phycocyanin at 84^{th} amino acid cysteine and altering the energy transfer in this cyanobacterium. Upon prolonged incubation β-subunit (22 kDa) is getting degraded due to the induction of proteases by mercury toxicity.

Lecture

Molecular chaperones and stress tolerance in cyanobacteria: Role of chaperone paralogs/cognates in the evolution of cyanobacteria

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A variety of environmental stress conditions including sudden temperature increase induce the transient synthesis of molecular chaperones that prevent non-specific protein aggregation and promote efficient folding of non-native proteins. They play a role not only under stress, but also under normal conditions. Molecular chaperone is defined as a protein that interacts with, stabilizes or helps another protein to acquire its functionally active conformation, without being present in its final structure. Different classes of structurally unrelated chaperones exist in cells. In general, each chaperone does not act on its own, but forms networks of collaboration with other chaperones.

During the course of evolution cyanobacteria have adapted to a vast range of environments. I assume that molecular chaperones, very ancient proteins, have played a key role in the adaptation since proteins are relatively unstable cellular macromolecules that are susceptible to denaturation under various environmental stresses. Thus, we have been trying to understand how molecular chaperones are involved in cyanobacterial defense against stress at the molecular level.

In contrast to *E. coli* that is the model organism for chaperone studies, cyanobacteria contain multiple genes encoding *groEL* and *clpB*. One of the members in each gene family appears to code for GroEL (chaperonin) or ClpB (Hsp104) whose structure and function are similar to the corresponding one in *E. coli*. However, the other member(s) codes for a chaperone(s) that is quite different from the other one and the *E. coli* homolog. Cyanobacteria as well as *E. coli* contain a diverse class of J-proteins such as DnaJ that functions with DnaK (Hsp70). J-proteins appear to be much more diversified in cyanobacteria than *E. coli*. I will show our data regarding differences between multiple paralogs/cognates of GroEL, ClpB, or J-protein in cyanobacteria and discuss possible benefits of the diversity for the cyanobacterial evolution.

Lecture

Long term exposure of high light induced changes in thylakoid organization and their photosynthetic parameters from *Chlamydomonas reinhardtii*

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Light is essential for all photosynthetic organisms but excess can leads to damage the photosystems. Here, we have grown *Chlamydomonas reinhardtii* cells in different high light intensities to understand the thylakoid organisation and photosynthetic function. We observed faster growth and more biomass production in 500 µmol m^{-2} s^{-1} light intensity while reduced in high light (1000 µmol m^{-2} s^{-1}). The pigment content gradually decreased (Chl *a*/*b* ratio), higher content of lutein, neoxanthin and loroxanthin at 500 µmol m^{-2} s^{-1} while there were reduced in 1000 µmol m^{-2} s^{-1}. Surprisingly, violaxanthin and β-carotene are reduced in both 500 µmol m^{-2} s^{-1} and 1000 µmol m^{-2} s^{-1} light intensities and indicates that non-photochemical quenching has been increased due to photoprotection. Additionally, drastic changes in photosynthetic parameters demonstrates low photochemical yield from high light grown cells. Further, reduction in rate of oxygen evolution indicates low photosynthetic activity for high light acclimated cells. Additionally, protein contents of D1, D2, LHC II subunits, CP43, CP47 etc. were reduced. In turn, the supercomplexes formation was dramatically changed in high light condition and this may be due to change in protein-pigment complexes which are in agreement with protein profile. Moreover, sucrose density gradient showed the high light effect was mainly affected the core complexes as compared to LHCs in high light acclimatized cells. Further, CD spectra of high light acclimatized cells showed decrease in magnitude of psi-type bands that indicates ordered arrays of PSII–LHCII super-complexes was altered. These results specify that acclimation to high light stress with photo-protective mechanisms by changes in thylakoid protein profile that leads to low photochemical yield.

Lecture

Toxin-antitoxin mediated programmed cell death in the cyanobacterium *Synechocystis*

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Two putative heat responsive transcriptional regulators, Ssl2245 and Sll1130 encoded by a dicistronic operon, behave similar to that of bacterial toxin-antitoxin systems. Similarity search indicated that the Ssl2245 protein is an AbrB like transcription factor and the Sll1130 is a putative *pemK* family transcriptional regulator. Targeted inactivation of Δ*ssl2245* and Δ*sll1130*, and subsequent gene expression profiling, revealed that their inactivation resulted in induced expressions of several heat shock genes and uncharacterized plasmid encoding genes. The Δ*sll1130* mutant cells exhibited enhanced heat tolerance and increased pilli formation when compared to wild type cells. The *sll1130* and *ssl2245* genes are constitutively expressed and the corresponding proteins are always present in the cell as a hetero-multimeric protein complex. The Sll1130 is an endonuclease, degraded RNA upon incubation of cells at high temperature. The endoribonuclease activity of Sll1130 has been inhibited by tight association of the HcdI at optimal growth temperature. These proteins exhibit differential stability, HcdI being less stable and forms large aggregates at high temperature. The Ssl2245 protein gets dissociated from Sll1130 at high temperature, allowing HcdN to cause heat induced death of *Synechocystis* cells. Collectively, Ssl2245-Sll1130 mediate heat induced programmed cell death of *Synechocystis*. The discussion will be focussed on how these proteins mediate heat induced programmed cell death in *Synechocystis.*

Lecture

AhpC (alr 4404) confers abiotic stress tolerance in cyanobacteria by modulating photosynthesis and antioxidative protein network

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AhpC, 1-cys peroxiredoxin, distributed across living organisms, is an antioxidant protein offering protection from reactive oxygen species. Its biochemical and molecular characterization following heterologous expression in *E. coli* demonstrated cross tolerance to a host of abiotic stress. Its transgression in the cyanobacterium produced enormous change in the proteome. A comparative proteomics of ahpC-over expressing (AnFPNahpC), ahpC mutant (ΔahpC) and wild type control *Anabaena* PCC 7120 unveiled AhpC-triggered two major events: (i) fold increase in proteins of metabolically most significant variables e.g. nitrogen fixation (1.6), photosynthesis (PSI, 1.08; PSII, 2.137), respiration (5.66) in AnFPNahpC and their (nitrogen fixation, PSI) PSII, respiration) down regulation in ΔahpC as compared to control cells, and (ii) appreciable upregulation of antioxidative defense proteins and their subsequent down regulation in ΔahpC indicates the regulatory function of ahpC which was further attested by string network. where ahpC specifically interacts with upregulated GroEL (2.04) and SODA (1.32 fold) of AnFPNahpC. These proteins were down regulated in the ΔahpC as compared to the wild type. In view of enhance nitrogen fixation, photosynthesis and tolerance to a host of abiotic stress the transgenic *Anabaena* holds potential for application in rice fields.

Lecture

Cross-talk of chloroplasts with mitochondria and peroxisomes: Mitochondrial redox is a major signal to mediate the interactions

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Chloroplasts and mitochondria are not as autonomous as they were thought to be. The function of chloroplasts is strongly dependent on mitochondria as well as peroxisomes, besides cytosol. Mitochondrial respiration and photorespiration are essential for optimizing photosynthesis, and to protect against photoinhibition imposed by excess light. The interplay of these three pathways is facilitated by two major phenomena: sharing of energy/metabolite resources and maintenance of optimal reactive oxygen species (ROS) levels. The responsibility of generating the cellular requirements of ATP and NAD(P)H is mostly by the chloroplasts and mitochondria. In turn, besides the chloroplasts, the mitochondria, cytosol and peroxisomes are common sinks for reduced equivalents. It is imperative that the redox in the cellular compartments of chloroplasts, mitochondria, peroxisomes or cytosol exhibits a strong influence. Although the metabolic interactions between mitochondria and chloroplasts are extensively examined, the redox basis of cross-talk between mitochondria, chloroplasts and peroxisomes is not much studied.

We have been studying the consequences of the modulation of mitochondrial metabolism by either inhibitors or mutants, on peroxisomal components. We could trigger ROS production in different compartments of the cell with compounds, such as paraquat (chloroplast); menadione (mitochondria) and abscisic acid (plasma membrane). We have examined the characteristics of three peroxisomal enzymes: catalase, glycolate oxidase and hydroxypyruvate reductase. The key metabolites related to photorespiration also were determined under redox modulation. My talk would emphasize the beneficial interactions among photosynthesis, dark respiration and photorespiration, in relation to redox modulation. The intracellular ROS levels appear to be a major signal to modulate and mediate the cross-talk between the organelles of metabolism of chloroplasts, mitochondria and peroxisomes.

Lecture

A proteomic-based insight into the role of pod wall in regulating carbon allocation and seed filling in soybean under potassium iodide-simulated terminal drought stress

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Water limitation during the reproductive phase of plants is highly detrimental leading to significant reduction in yields. Simultaneous regulation of photosynthesis and photo-assimilate allocation to competing sinks becomes crucial for sustaining final yield under such adverse conditions. Despite the present scientific understanding on drought-responses, it is still highly challenging to develop "smart" crops, which can sense water-deficit at the very onset and respond immediately for regulating the resource allocation to reproductive structures. It is recently known that pod-wall (pericarp) is not merely a protecting structure but also plays a crucial role in regulating carbon partitioning during seed filling under various environmental cues. In the present study, we analysed the soybean pod wall proteome response and also the mRNA expression patterns of sucrose metabolizing enzymes, sugar transporters and cell cycle regulating proteins within 24 h of potassium iodide (KI)-induced desiccation, which effectively simulated the initial onset of drought conditions at R5-5.5 stage of soybean pod development. Within 24 h of KI-treatment the sucrose synthase, sucrose-H^+ symporter, SWEET24 sugar efflux protein, SNF1 related kinase, cyclinD and cyclin dependent protein kinase gene expressions were significantly induced. Pod wall proteome showed reproducible up-regulation of some of the key regulatory and oxidative stress related proteins including, 14-3-3 protein 2, rubisco activase, ATP synthase β subunit and heat shock protein 70, while some of the major proteins including adenosine kinase and 13S globulin seed storage protein remained unchanged. We also analysed the impact of stage-specific (R5, R5.5 and R6) KI-treatment on the final carbon allocation pattern in leaves, pod walls and seeds separately, which showed significant reduction in starch and reducing sugar accumulation in the leaves of KI-sprayed plants, but remained similar to controls in the pod wall and seeds of KI treated plants. Our data provide key insight into the role of pod wall in regulating carbon allocation during seed filling under initial onset of drought stress. The present study is highly crucial for understanding the initial regulatory aspects of seed filling in soybean under natural drought conditions.

Lecture

Controlling alternatives for photosynthetic electron flow to improve yield

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Photoautotrophic organisms such as plants and Cyanobacteria use visible light to generate high-potential reductant such as reduced ferredoxin or NADPH. At the same time, during photosynthetic electron flow, using the proton gradient across the thylakoid membrane, ATP as universal energy equivalent is continuously formed. Depending on the endergonic metabolic reactions that consume reductant and ATP, the rates of formation of each of them and their ratios need to be adjusted in each cellular compartment. Multiple indirect shuttle systems link the various sites of production and consumption of ATP and NAD(P)H. Any imbalance will immediately lead to uncontrolled formation of reactive oxygen species and damage. This is particularly of importance when the environmental conditions fluctuate and the acceptors of biochemical energy and reducing power are not available at all the times. Therefore, alternative electron acceptors along the path of electron flow, and uncoupling of electron flow from ATP synthesis are required. These energy exits and valve systems need to be strictly controlled so that they are active only in the case of excess energy and reducing power. Among others, Mehler reaction, malate valve and alternative oxidase can optimize the interplay of biochemical reactions and product formation. The molecular characteristics of these poising systems and the regulatory principles that govern the operation of these supportive systems will be presented. Such controls should be considered when increase of yield of any energy-containing product is aimed for in engineered systems or under challenging environmental conditions.

Lecture

Membrane transport in chloroplasts: optimising cell performance for adverse environmental conditions

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Sustainable food production is severely hampered by a range of abiotic stresses such as salinity, drought, heat, flooding costing agricultural sector over $120 billion p.a. in lost opportunities. The stress-induced decrease in plant productivity is ultimately related to its reduced capacity for CO_2 assimilation and is regulated at various levels of plant functional organization, from the whole-plant (e.g. stomatal limitation of photosynthesis) to tissue- and organelle-specific levels (e.g. stress-induced inhibition of the primary photosynthetic processes in chloroplasts). In this talk, I will use salinity stress as an example to understand how plants optimize their photosynthetic performance under adverse soil conditions by optimizing membrane-transport processes across the plasma membrane and cellular organelles. By comparing halophyte and glycophytes species I show that halophytes, naturally salt-tolerant species, can overcome stress-induced stomatal limitation by switching to CO_2 concentrating mechanisms and increase the number of chloroplasts per cell under saline conditions and, at the same time, optimising water use efficiency by reducing their stomata density. I then show that salt entry into the stroma may be critical for grana formation and PSII activity in halophytes but not in glycophytes, and inhibition of some stromal enzymes by salt is significantly lower in halophyte species. I show that halophytes accumulate much more chloride in chloroplasts than glycophytes and use sodium in functional roles in this organelle. I then discuss the molecular identities of candidate transporters that move sodium, chloride and potassium across envelope and thylakoid membranes and discuss how their operation may regulate chloroplast membrane potential, stromal pH and osmotic relations in chloroplasts, ultimately affecting leaf photochemistry and PSI and II activity.

Lecture

Cu nanoparticles and bulk Copper have different mechanism to affect growth and photosynthesis in rice plants

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Rice plants were grown hydroponically in CuONP (<100 nm size) or bulk Cu ($CuSO_4$) in 0–1000 mg L^{-1} containing Hoagland medium to study comparative biochemical behaviour. It was observed that accumulation of Cu was many fold higher in roots and the accumulation was mostly in non-ionic form in plants treated with CuONP in comparison to plants treated to bulk Cu where the accumulation was in the form of metal ions. Toxicity threshold for the CuO NP and bulk Cu was observed to be 100 and 10 mg L^{-1} concentration respectively. No oxidative damage was observed by CuONP but observed in plants treated with bulk Cu. However, growth and photosynthesis (photo-phosphorylation as well as CO_2 fixation) measured using IRGA and chlorophyll fluorescence showed significant decline due to both the treatment. TEM analysis showed accumulation of CuONP in the chloroplasts resulting in destacking and distortion of thylakoids whereas bulk Cu has no such effect on thylakoid membranes. It is proposed that CuONP attributed toxicity to growth and photosynthesis is due to the effect of accumulation of metal nano particle and not Cu ions in chloroplasts resulting in structural changes in thylakoid membranes whereas toxicity effect of bulk Cu was due to Cu ions mediated oxidative damage due to generation of ROS.

Lecture

Enhanced photosynthetic carbon assimilation and antioxidative efficacy favoured sustained growth of drought stressed Pigeonpea under elevated CO_2

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Elevated CO_2 influences crop responses to drought stress depending on the photosynthetic behaviour of the plant. In the present study, Pigeonpea (*Cajanus cajan* L.), a potential legume food crop was assessed for its photosynthetic physiology, antioxidative system as well as C and N metabolism with elevated CO_2 and drought interaction. Pigeonpea was grown in open top chambers under elevated CO_2 (600 µmol mol^{-1}) and ambient CO_2 (400 µmol mol^{-1}) concentrations which was later subjected to drought stress by complete water withholding. The drought stressed plants were re-watered to gain normal physiological growth and assessed the recoverable capacity in both elevated and ambient CO_2. We assessed the photosynthetic physiology including photosystem II efficiency, growth and biochemical responses in addition to antioxidative responses in Pigeonpea. The elevated CO_2 grown Pigeonpea showed greater gas exchange physiology, nodule mass and total dry biomass over ambient CO_2 grown plants under drought stress albeit a decrease in leaf relative water content (LRWC). The higher C assimilation resulted in increased carbohydrate accumulation in elevated CO_2 grown plants which were transported to sink tissues effectively. Glucose, fructose and sucrose levels were measured to understand the role of hexose to sucrose ratios (H:S) in modulating the drought responses. Free amino acids levels, total N and protein contents as indicatives of N assimilation provided insights into C and N balance under drought and CO_2 interactions. The enzymatic and non-enzymatic antioxidants showed significant changes in drought stressed Pigeonpea under elevated CO_2 thereby protecting the plant from oxidative damage. Our results clearly demonstrated the protective role of elevated CO_2 under drought stress at lower LRWC and gained comparative advantage of mitigating the drought stress induced damage over ambient grown Pigeonpea plants.

Lecture

Electrical responses and photosynthesis in higher plants: a theoretical problem and practical perspectives

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Electrical responses (ERs) are transitory changes in the electrical gradient across the plasma membrane. In plants, the changes can be induced by various stimuli including non-optimal temperatures, mechanical irritations, excess light, changes in water regime, injuries by insects, etc. ERs include electrical signals (ESs), which propagate from the stimulated zone to intact parts of the plant body, and local electrical responses (LERs), which are generated in the stimulated zone. It is probably that the main role of ESs (action potentials, variation potentials, and system potentials) in higher plants is participation in development of system responses under spatially heterogeneous action of stressors. Influence of ESs on photosynthesis includes two aspects. (i) ESs induces changes in photosynthetic activity; in particular, they inactivate photosynthetic dark reactions and a linear electron flow in chloroplasts and stimulate a non-photochemical quenching of fluorescence and a cyclic electron flow around photosystem I. The inactivation is connected with ESs-accompanied influxes of Ca^{2+} and H^+; production of ROS and abscisic and jasmonic acids is possible to participate in the process, too. (ii) ESs increase tolerance of the photosynthetic machinery to stressors including low and high temperatures (decrease of damage of photosynthetic processes and increase of their reparation). The increased tolerance can be connected with activation of the cyclic electron flow, stimulation of the non-photochemical quenching, and increase of the ATP content in leaves. Connection of LERs (receptor potentials, voltage transients, and local action potentials) with photosynthesis is weakly investigated; however, similarity between ionic mechanisms of ESs and LERs is argument to support the connection. In particular, heating-induced LERs can be strongly connected with residual photosynthetic activity after thermal stress; it is possible that this effect is caused by positive influence of LERs on photosynthetic thermotolerance. Connection of ERs and photosynthesis in higher plants opens important practical perspectives. (i) Regulation of electrical signaling (regulation of a threshold of ERs and other parameters) by chemical agents and/or by genetic transformation for a control of crop under environmental stressors (control of photosynthetic activity and its tolerance). (ii) Development of new methods of fast and remote diagnostics of biotic and abiotic damages. It is known that different stressors can induce different electrical responses in plants and, thereby, photosynthetic changes with different spatio-temporal distribution. Revealing specific distributions for action of different stressors can be basic for this diagnostics.

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Lecture

Chloroplast and mitochondrial interactions: possible roles of nitric oxide and reactive oxygen species in mesophyll protoplasts of pea (*Pisum sativum*)

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Plants are subjected to diverse stresses due to the environmental constrains, which can lead to oxidative damage at the cellular level. Increased production of reactive oxygen species (ROS) and/or nitric oxide (NO) is common under such stress conditions. These ROS and NO can either damage the cellular metabolism or acts as signaling molecules. We attempted to examine the consequences of exogenous application of NO (in the form of SNP) and H_2O on the patterns of photosynthesis and respiration in mesophyll protoplasts of pea plants. Elevated levels of NO (released by SNP) severely inhibited the photosynthetic performance of mesophyll protoplasts, while exhibiting marginal/no effect on dark respiration. NO affected the efficiency of photochemical reactions of chloroplasts, particularly electron transport, as indicated by Chlorophyll *a* fluorescence (OJIP) transients and O_2 evolution data. In contrast to the effects of NO, the exogenous application of H_2O_2 resulted in drastic decrease in the rates of respiration, with only a marginal effect on photosynthesis. At the concentration used in our study, H_2O_2 didn't affect the electron transport activities of PS I or PS II. We conclude that chloroplasts are the primary targets of NO and mitochondria for H_2O_2 in plant cells. We then attempted to examine, if NO and ROS can mediate the interactions between photosynthesis and respiration. In presence of NO, the sensitivity of photosynthesis, to either antimycin A or SHAM didn't alter much. However, H_2O_2 decreased the sensitivity of photosynthesis to mitochondrial inhibitors, particularly SHAM. We suggest that H_2O_2 could possibly modulate the crosstalk between chloroplasts and mitochondria.

Lecture

Chloroplastic ATP synthase modulates H^+-gradient across the thylakoid membranes for preventing photosystem I photoinhibition in higher plants

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Over-reduction of photosystem I (PSI) stimulates the reactive oxygen species (ROS) production during photosynthesis. ROS cause PSI photoinhibition and suppress CO_2 fixation reaction. Therefore, to prevent ROS production in PSI and PSI photoinhibition is important to sustain photosynthesis in chloroplasts.

For preventing over-reduction of PSI, H^+-gradient across the thylakoid membranes (ΔpH) contributes to oxidizing PSI during photosynthesis [1]. However, the exact regulatory system to modulate ΔpH during photosynthesis has remained to be clarified.

In this study, we aimed to identify the critical factor to modulate ΔpH during photosynthesis by screening ethyl methane sulfonate (EMS)-treated *Arabidopsis thaliana*, in which the formation of ΔpH is impaired and the PET chain caused over-reduction under illumination. Then, we isolated allelic mutant that carries a mis-sense mutation in the γ-subunit of chloroplastic CF_0CF_1-ATP synthase. We also found that this mutant impaired ΔpH formation because of the higher H^+-efflux conductance from lumenal side to stromal side in chloroplastic CF_0CF_1-ATP synthase.

Here, we discuss the importance of ΔpH management by chloroplastic CF_0CF_1-ATP synthase during photosynthesis to avoid over-reduction of PSI and PSI photoinhibition.

1. Tikhonov, A.N. (2013) Photosynth. Res. 116, 511–534

Lecture

Photoinhibition of photosystem I provides protection from excess electron transfer to molecular oxygen and accelerate dissipation of excess absorbed energy

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Recent years have revealed the vulnerability of photosystem I (PSI) under fluctuating light conditions [1, 2] occurring not only under the laboratory conditions but also in the field. This has raised questions and discussion about the mechanism(s) of PSI damage and how it is related to the reactive oxygen species (ROS) production in the thylakoid membrane. To this end, we have investigated superoxide ($O_2^{\bullet-}$) production in the wild type (WT) and the *pgr5* mutant of Arabidopsis, which are differentially susceptible to PSI photoinhibition upon high light treatments [3]. Using specific inhibitors of electron transfer at the plastoquinone, cytochrome b_6f and plastocyanin sites, we show PSI as an exclusive site of $O_2^{\bullet-}$ production in the thylakoid membrane. Two clearly distinct sites were identified for production of $O_2^{\bullet-}$ in PSI. One site is independent and the other one dependent on the functionality of the iron-sulphur (FeS)-clusters. Exposure of WT and *pgr5* mutant plants to changing light intensities, inducing a damage of FeS-clusters (F_A and F_B), concomitantly decreased the capacity of $O_2^{\bullet-}$ production in PSI. We suggested that the F_X clusters do not directly participate in $O_2^{\bullet-}$ production. A decline in ROS production and an increased energy dissipation following PSI photinhibition provide evidence for a new concept and physiological role of PSI photoinhibition under natural conditions. The damage of the F_A and F_B clusters serve as a photoprotection mechanism by limiting excess ROS accumulation in the stroma.

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Poster

Light saturation kinetics of photosynthetic parameters in *Pisum sativum* L. seedlings grown under different wavelengths of light

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Photosynthesis is the fundamental process for primary productivity on earth and gives food security for human civilization.

Objective: The efficiency of primary photochemical reaction of photosynthesis is studied using PAM fluorimetry in *Pisum sativum* L. grown under different light qualities (wavelengths). The photosynthetic parameters for photochemical and non-photochemical efficiency are derived to elucidate the impact of different wavelengths of light on these parameters.

Materials and methods: Pea (*Pisum sativum* L.) seedlings were grown hydroponically in 0.5N Hoagland solution under 12 h photoperiod with uniform LED illumination (135 µmol m^{-2} s^{-1}) of white (400–700 nm), red light (600–700 nm), and blue light (400–500 nm). The photosynthetic parameter of the fully grown leaves at the 3rd node on 7th, 14th and 21st day was measured with a Dual-PAM-100 fluorimeter. The quantum efficiency of photosynthesis (qP), quantum yield (ΦII), electron transport rate (ETR), and non photochemical quenching (NPQ) parameters were studied as described by Misra et al. (2012) and Kalaji et al. (2014).

Results: The Φ(II) and ETR values of plants grown under white light was maximum, compared to blue or red light, indicating that the white light treatment is the most helpful for improving the photoreaction rate of the PSII reaction centers. The light saturation kinetics for qP shows a decrease with an increase in the actinic light, suggesting that the PSII centers get closed with increase in irradiance and this activity increased with seedling age in blue light grown plants but it decreased in red light grown plants. The action of blue light on the light saturation response for qP is similar and at par to white light. Kinetics of NPQ indicated that the light absorbed by the plant's antenna pigments was dissipated as heat to a greater extent in the blue light grown plants. To the contrary, NPQ values for plants grown under the red LED was the lowest, compared to both blue and white light.

Poster

Modification in light utilization efficiency by *Asteracys quadricellulares* at varying light intensities under mixotrophic regimes

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High light intensities may depreciate the growth rates and overall productivity of microalgae in outdoor cultivation. With this aspect in mind, a study was designed to understand the extent of light limitation in cells of *Asteracys quadricellulares* adapted and grown at varying light intensities (180–1000 μE). Additionally, this study explored the role of external carbon (glucose) in the media on the adaptability of microalgal cells to high light by measurement of the light saturation coefficient (I_k). Microalgae regulate light harvesting by photosystem II in response to changes in light intensity. The non-photochemical quenching measurement of chlorophyll fluorescence is a photoprotective mechanism adopted by the cells to regulate excess light. This study undertakes a detailed analysis of the relaxation of NPQ in differently adapted cells to see the effect of an external carbon source on the quenching mechanism.

Poster

Importance of Reactive Oxygen Species (ROS) and Nitric Oxide (NO) during stomatal closure by polyamines in *Arabidopsis thaliana*

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Gaseous exchange plays an important role in photosynthetic carbon assimilation. Stomata act as a important modulator of photosynthesis, as CO_2 enters the leaves through stomata. Opening and closing of stomata are the result of changes in the turgidity of constituent guard cells. Several abiotic and biotic factors induce stomatal closure. For e.g. abscisic acid (ABA), a "stress phytohormone" induces stomatal closure in different plant species through the concerted action of a plethora of signalling components, such as reactive oxygen species (ROS), reactive nitrogen species (NO), Ca^{2+}, cytosolic pH, sphingolipids, phospholipids and MAP kinases were all involved in ABA induced stomatal closure. Drought is known to induce an increase in polyamines (PAs), which are ubiquitous polycationic nitrogenous compounds, that are associated with plant adaptation to abiotic factors. It is imperative that these polyamines in turn restrict stomatal opening and conserve water loss. We have therefore studied the effect of three PAs: putrescine (Put), spermidine (Spd) and spermine (Spm), on stomatal movement in *Arabidopsis thaliana.* Among these, Put and Spd were more effective than Spm, in inducing stomatal closure. The levels of ROS and NO also enhanced in guard cells on exposure to the PAs, Modulators of ROS and NO revealed that these are essential for the stomatal closure. To find out the enzymatic sources of the ROS and NO, PAs induced stomatal closure was examined in mutants like *nia1/2* (deficient in nitrate reductase) *cuao1-1* (copper amine oxidase deficient) and *pao4* (lack of polyamine oxidase). The results indicated that ROS and NO are essential signalling components for PA-induced stomatal closure and NADPH oxidase and amine oxidase act as a source of ROS/NO during stomatal closure by PAs in *Arabidopsis thaliana*.

Poster

PSI and PSII electron transport in leaves of various isogenic *chlorina* wheat mutant lines in relation to photoprotection and photosynthetic capacity

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Antenna mutants represent a unique tool to study photosynthetic processes running at the level of the thylakoid membrane. In our experiments, we examined *in vivo* high light responses and photosynthetic capacity of chlorophyll *b*-less isogenic mutant line of spring wheat (*Triticum aestivum* L.) – ANBW-4A, ANBW-4B, ANK-32A and durum wheat (*Triticum durum* L.) – ANDW-7A, ANDW-7B, ANDW-8A, ANDW-8B in comparison to parental lines representing the wild type (WT) in different growth phases. The mutants differed significantly in chlorophyll content and growth rate. Whereas in initial growth phases, the chlorina-phenotype effect was dominant in all mutant lines, in later growth phases, the chlorophyll content in some mutants increased significantly, but remained at a significantly lower level compared to WT. The simultaneous measurements of chlorophyll fluorescence and P700 absorbance indicated altered electron and proton transport, resulting to lower trans-thylakoid pH-gradient, leading to lower NPQ in early growth phases. As a result of insufficient regulation of linear electron transport, the acceptor side of photosystem I (PS I) was more reduced, creating conditions for enhanced oxidative damage due to reactive oxygen species. The shift of balance between PSII and PSI redox poises indicates lower PS I to PS II ratio in chlorina mutants compared to WT. Our results also suggest that chlorina mutant ofwheat had lower capacity to increase the rate of cyclic electron flow around PSI, which makes these mutants more susceptible to environmental constraints. The severity of these effects varied in different mutants and growth phases. The relationships between leaf traits, photochemical responses and photosynthetic capacity were also examined.

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Poster

Mimicking effect of continuous irradiance and low pH on stacking pattern and energy distribution between two photosystems of thylakoids

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The photosynthetic organisms constantly face a transition between the light-harvesting and photo-protective states of the thylakoid membrane due to changes in their light environment and an attempt was made to unravel the regulation of chromatic adaptation under continuous irradiance mimicking natural day light. For this firstly, the energy distribution between photosystems (PSI & PSII) under continuous white light irradiance was assessed by monitoring the progress of their fluorescence emission (F_{PSI}/F_{PSII}) at 77 K. The observations indicated oscillation of F_{PSI}/F_{PSII} with the progress of irradiance treatments. Regulation of chromatic adaptation, also underlie structural rearrangements escorted by light-dependent reorganizations of the protein landscape (PSII-LHCII assembly) within the thylakoid membrane topography. Such light induced structural changes were then monitored by few spectroscopic and microscopic techniques. The observations revealed irradiance induced decrease in extent of stacking associated with reorganization within PSII-LHCII assembly. Then we unraveled the factors that are regulating the observed phenomena of chromatic adaptation. The trans-thylakoid ΔpH that is generated due to acidification of thylakoid lumen by light induced electron transport appeared to be an important determinant of the chromatic adaptation occurring under continuous white light irradiance. Furthermore reciprocal relationship existed between progress of F_{PSI}/F_{PSII} at 77 K and stacking arrangement of thylakoids following continuous white light exposure for initial ~30 minutes. Mimicking effect of lowering of thylakoid lumen pH (in absence of light) and continuous white light treatment had also been revealed.

Poster

Key player in grain filling of Indian wheat (*Triticum aestivum* L.): Spike photosynthesis

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Spike photosynthesis contributes majorly in grain filling in wheat by utilizing the the flag leaf and spike. Abiotic stress adversely effects the photosynthetic process and thus decrease in the grain yield. In order to unravel the role of flag leaf, awn, and spike in wheat grain filling and spike photosynthesis, thousand kernel weight were measured after removing flag leaves, awns, and by shading the spike in four wheat genotypes (PBW343, C306, K7903, HD2329) for two seasons (2014–2015, 2015–2016). A significant decrease in the grain filling was observed for all the genotypes. These results indicate the role of these organs contributing spike photosynthesis and influencing grain filling. The role of the awn tissue was investigated in PBW343 for its role in spike photosynthesis during heat stress. Deep transcriptome sequencing of the awn tissue (PBW343) was performed and it revealed 147573 unigenes. Out of these, 394 genes were differentially expressed genes (DEGs). These DEGs constitutes 201 upregulated and 193 downregulated genes. Genes involved in photosynthesis (Ribulose bisphosphate carboxylase/oxygenase activase B, NADH dehydrogenase, Fe-S protein2), membrane integrity (ATP-dependent zinc metalloprotease FTSH6), and ion channel transporters (two-pore potassium channel3) were prominently expressed. Gene Ontology (GO) enrichment analysis represents PSII associated light-harvesting complex II catabolism, chloroplast organization, photosynthesis light harvesting in photosystemI, ethylene biosynthesis, regulation of oxidoreductase activity, stomatal closure, chlorophyll biosynthesis categories, which are highly overrepresented under heat stress conditions. Therefore, utilizing the awn transcriptome information, Rubisco activase (RCA) gene was chosen for overexpression studies in wheat and rice with the aim to enhance the photosynthetic efficiency of the spike tissue leading to higher grain filling.

Poster

Water-stress induced downsizing of light-harvesting system protects developing seedlings from photo-oxidative damage

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To understand the impact of water-stress during early photomorphogenesis, polyethylene glycol 6000 was applied to the roots of 5-day-old etiolated rice (*Oryza sativa*) seedlings for 16 h and illuminated up to 72 h. In consonance with reduced synthesis of chlorophylls, light-harvesting chlorophyll-proteins Lhcb1, Lhcb2, Lhca1 and Lhca4 were down-regulated. Photosynthetic proteins of PSII i.e., Cytb559, oxygen evolving complex proteins, OEC16, OEC23 and OEC33; and that of PSI i.e., PSI-III, PSI-V, PSI-VI decreased in abundance resulting in reduced light absorption by antennae and utilization by reaction centers. Consequently, light-limited and light-saturated electron transport rates of PSII and PSI were reduced by 55% and 25% respectively. The variable/maximum fluorescence and quantum yield of PSII declined. The 77 K fluorescence emission spectra demonstrated an alteration in the structural organization of thylakoid membranes due to the loss of LHCI. Salt (Mg^{2+})-induced grana stacking was impaired. Proteomic analysis revealed the down-regulation of proteins involved in light reaction, carbon reduction reaction, protein folding, energy balance, cell homeostasis, and up-regulation of antioxidative enzymes. Results demonstrate that unlike water-stressed mature plants, developing seedlings under water stress conditions could downsize their light-harvesting capacity and associated components of photosynthetic apparatus to prevent excess ROS generation and membrane lipid peroxidation.

Poster

Investigations on molecular mechanism of mercury detoxification involving a *merR* family transcription factor in photosynthetic cyanobacterium *Synechocystis* PCC6803

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Some bacteria are resistant to heavy metals and are capable of growing even at high concentrations of some heavy metals. Whereas, some metal ions are important for the cell at low concentrations and lethal at high concentrations. Microorganisms use different strategies to regulate metal homeostasis, such as sequestration and accumulation as storage bodies and regulated transport. Mercury (Hg) is one of the highly toxic metal that severely damage both light and dark reactions in photosynthetic bacteria. However, some bacteria use MerA, mercury reductase, a flavoenzyme that reduces Hg (II) to the volatile Hg (0) form. *merR* a transcription factor is a regulator of *merA* gene. *slr0701*, a putative transcriptional regulator in *Synechocystis* is predicted to function as regulator of mercury detoxifying genes. The open reading frame, *slr0701* was inactivated by targeted mutagenesis. The wild type *Synechocystis* cells and the Δ*slr0701* showed no significant difference in growth at optimal conditions, but in the presence of $HgCl_2$ Δ*slr0701*, exhibited a slow growth phenotype and became sensitive to $HgCl_2$. *In silico* analysis of Slr0701 protein showed that N-terminal DNA binding region is well conserved in bacteria. Wild type *Synechocystis* cells showed induced expression of *slr0701 (merR)* and *slr1849 (merA)* when 500 nM mercury chloride treatment was given. However, the induced expression of *slr0701* and *slr1849* transcripts were not observed in the Δ*slr0701* mutant upon mercury treatment, suggests Slr0701 regulates the *merA*, mercury reductase as well as regulation of its own gene expression. Purified Slr0701 protein binds to the upstream operator region of its own gene and autoregulate its expression. The mechanism of gene regulation and mercury detoxification, involving Slr0701 will be discussed.

Poster

Study on shuttling of light-harvesting complexes upon state transition by using cryogenic optical microscope

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Photosystem I (PSI) and Photosystem II (PSII) play central roles in the photochemical reactions in photosynthesis. Generally, PSI and PSII are located in stroma lamella and grana stack (diameter 0.3 um), respectively. A mechanism called state transition (ST) is known as a function to control the excitation balance between the PSs *in vivo*, which assures an efficient photosynthesis. Although it has been believed that ST is caused by shuttles of light harvesting complex of PSII (LHCII) from PSII (ST1) to PSI (ST2) (model0), there has been no direct observation of its movement within a cell. Here, we examined the movement of LHCII by using a newly developed cryogenic microscope [1]. In this microscope, it is possible to observe cells at 77 K with a high-resolution objective lens (NA0.9, l00, Mitutoyo). We have developed the laser-scanning confocal optical system based on this microscope which enables observation of a fluorescence spectrum at each pixel. The system provides the lateral and axial resolutions of about 0.3 mm and 1.2 mm, respectively. At 77 K, a fluorescence spectrum of chloroplast can be divided into PSI, PSII, and LHCII components. Thus, this system makes it possible to obtain the intracellular distributions of these components. In this experiment, we used *Chlamydomonas reinhardtii* incubated on TAP medium as samples. After the ST induction of the cells, we cooled those at 77 K and obtained fluorescence images. From this measurement, we could observe the changes in the intracellular distribution of LHCII upon ST for the first time. The result showed that brighter fluorescence of LHCII was observed near PSII than PSI in both ST1 and ST2. On the other hand, co-existence of the LHCII and PSII emission was suppressed for cells showing intermediate PSII/PSI emission peak ratio. According to the above observation, we propose a modified ST model.

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Poster

Plant profiling of photosynthetic activity in leaves of two ecotypes *Platanus orientalis* L. subjected to moderately high temperatures

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Plant leaf profiling by analysis of photosynthetic machinery in leaves of different physiological age in *Platanus orientalis* plants belonging to Bulgarian and Italian ecotypes was performed with chlorophyll fluorescence method. Temperature treatment was carried out in controlled conditions in a phytostatic chamber for 3 days (4 hours at 38°C or 41°C per day), followed by 3 days of recovery at 25°C. The physiological state of leaves of different age was analyzed *in vivo* by studying the changes in the prompt chlorophyll fluorescence transients. The changes occurring in the photosynthetic apparatus during senescence of the plant cells were realized in different ways: the Italian ecotype showed ungrouping of the PS2 antenna complexes and suppression of the Oxygen Evolving System, while in the Bulgarian ecotype the opposite effects were found. At the same time, the photosynthetic apparatus of the two ecotypes was characterized by increase of the intersystem electron carrier number with leaf cell age. We could speculate that the processes of ontogenetic development and the senescence of the plant cell in the leaves of both ecotypes occurred at different rates (in the Bulgarian ecotype aging was delayed) which was probably predetermined by a different stress response at treatment with moderately high temperatures. We found that treatment with elevated temperatures induced changes in photosynthetic machinery more expressed for 41°C than for 38°C and dependent both on leaf age and on the plant ecotype.

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Poster

Alterations in photosynthetic pigments and antioxidant defense systems in wheat varieties subjected to a long-term drought stress followed by recovery

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Water is a fundamental constituent of plants for maintaining leaf structure and shape, photosynthesis, and thermal regulation. The study of the adaptation to drought and mechanisms of tolerance, the assessment of water deficiency are the actual tasks of the modern researches. Watering of bread wheat (*Triticum aestivum* L.) genotypes (Gobustan and Tale 38) was resumed after a long term soil drought. After 3 and 7 days of the resumption of watering, RWC, amounts of photosynthetic pigments (Chl*a*, Chl*b*, Car), reduced glutathione (GSH) and the activity of glutathione reductase (GR) were measured for the comparative study of watered and drought variants.

Water loss was found to be less in the leaves of the tolerant variety Gobustan compared with sensitive varieties. When the watering resumed RWC increased in both genotypes and approached to the control. Amounts of photosynthetic pigments decreased in both genotypes during drought and this decrease was sharper in the sensitive variety. Amounts of carotenoids did not change significantly in the watered variant of the Gobustan variety, whereas 17% increase occurred in Tale 38. Amounts of reduced glutathione decreased in both genotypes under drought. The activity of glutathione reductase increased significantly in the sensitive genotype compared with the tolerant genotype and remained at the high level after the resumed watering. This fact shows that the antioxidant defense system takes an active part in the protection of sulfonyl groups from the oxidation in leaf cells.

The obtained results showed that antioxidant defense system and photosynthetic apparatus function quite efficiently under drought. Both genotypes differing in their response to water deficiency preserved ability to recover after rehydratation.

Moreover, agronomic traits comprising flag leaf angle (FLAN), flag leaf width (FLW), flag leaf length (FLL), the ratio of length/width of flag leaf (FLR), flag leaf area (FLA) were measured. These parameters changed less in the tolerant Gobustan genotype under the influence of drought stress.

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Poster

Sulfur-mediated control of salinity impact on photosynthesis and growth in *Vigna radiata* L. cultivars involves glutathione and proline metabolism

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This study aimed to assess the response of two mung bean (*Vigna radiata* L.) cultivars Punt mung and Samrat to 50 mM NaCl stress and also to evaluate the role of sulfur (S; 1.0 mM SO_4^{2-} and 2.0 mM SO_4^{2-}) in alleviation of salinity stress. The response of photosynthetic characteristics (net photosynthesis, intercellular CO_2, stomatal conductance, rubisco activity), plant dry mass, content of proline, cysteine and reduced glutathione (GSH), and the activity of glutathione reductase were higher in the cultivar Punt mung than the Samrat under salinity stress, showing greater ability of Punt mung to resist salinity stress. In contrast, Samrat exhibited higher content of glucose and activity of proline oxidase. The effects of 2.0 mM SO_4^{2-} in alleviating salinity stress on photosynthesis and growth were more conspicuous on Puntmung. The higher tolerance of Punt mung to NaCl stress was a result of better difference in the maintenance of 2.0 mM SO_4^{2-}-mediated higher contents of cysteine, GSH and proline; higher activity of GR, but decreased activity of proline oxidase and content of glucose.

Poster

Damage of photosynthetic apparatus by anthracene is protected by *Bacillus subtilis* strain

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The immense industrialization and urbanization in developing countries is increasing pollution load on environment by releasing different chemical waste. Petroleum and oil industries release contaminants which contain toxic PAHs (Polycyclic aromatic hydrocarbons). The aim of this study was to investigate the effect of Anthracene (ANT) on photosynthesis of wheat plants and potential of microbes for degradation of ANT. Measurements were performed with 200 µM anthracene. Chlorophyll content and Chlorophyll *a* (Chl *a*) fluorescence transient were recorded and analyzed according to OJIP test. Inhibition of photosynthetic efficiency was observed after 30 days of ANT application. ANT toxicity lead to decline in chlorophyll content, quantum efficiency of PSII photochemistry, damage in water splitting complex, decline in performance index of PSII. ANT toxicity also showed decrease in light absorption, trapping and electron transport rate. In the presence of *Bacillus subtilis* strain less inhibition of photosynthetic apparatus was observed. Amount of ANT quantified using HPLC (High performance liquid chromatography), about 75% reduction in ANT was observed upon application of *Bacillus subtilis* strain.

Poster

The importance of reduction-induced suppression of electron flow (RISE) in aquatic plants: How do aquatic plants oxidize P700 under water?

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Natural sunlight exceeds the demand of photosynthesis so greatly that it can cause plants to produce reactive oxygen species (ROS) in photosystem I (PSI), which subsequently cause photo-oxidative damage. Recently, we have shown that plants actively maintain the reaction center chlorophyll of PSI (P700) oxidized under excessive light conditions to alleviate the production of ROS.

The oxidation of P700 is controlled by both the electron acceptor and donor sides of PSI. On the acceptor side, O_2-dependent alternative electron flow (AEF) such as flavodiiron protein (FLV) in cyanobacteria [1, 2] and photorespiration in C3 plants [3, 4] contribute to P700 oxidation as electron sink. On the donor side, ΔpH, which limits the electron transport in Cyt b_6f, and reduction-induced suppression of electron flow (RISE), in which the Q cycle in Cyt b_6f is suppressed [5], contribute to P700 oxidation.

The recent study has suggested that under suppressed photosynthesis conditions such as under water, AEF is driven by FLV not photorespiration [6]. However, aquatic plants, which don't have FLV, grow under water. Additionally, we showed the lower activity of photorespiration in aquatic plants than land plants. From these facts, we hypothesized that in aquatic plants, the P700 oxidation is mainly controlled by donor side not accepter side. In the present study, we aimed to reveal the P700 oxidation system of donor side in aquatic plants.

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Poster

Salt-stress induced changes in the supramolecular organization of the photosynthetic membranes of *Chlamydomonas reinhardtii*

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Accumulating evidence indicates that photosynthetic supercomplexes undergo supramolecular reorganizations within a short time frame during acclimation to abiotic stresses. These reorganizations include state transitions that balance the excitation energy between the two photosystems (PSs), thermal energy dissipation at energy-quenching sites within the light-harvesting antenna complexes (LHCs), and change between linear and cyclic electron flow. Adaptation to salt stress is highly important since every continent is affected by salinized soil and water. Therefore, we have studied the effect of salt stress on the supramolecular organization of photosynthetic complexes in the green alga *Chlamydomonas reinhardtii,* wild-type (WT) and two mutant strains: *stt7* – incapable of state transitions and *pgrl1* – deficient in Pgrl1 protein that is involved in the regulation of cyclic electron flow around PSI. We analysed the physiological effects of salt treatment (0, 50, 100 and 150 mM) using various biophysical techniques.

Our 77 K fluorescence spectroscopy measurements revealed that the PSII fluorescence emission intensity gradually decreased with increasing salinity, even moderate NaCl concentrations (50 mM) induced significant reduction, while emission from PSI remained stable. At the same time, increased emission in the 670–680 nm range indicated the presence of uncoupled LHCII. The fluorescence kinetic measurements by time-resolved fluorescence spectroscopy, showed that the average lifetime of PSII component was longer at high salinity due to the appearance of a long-lived (1–3 ns) decay component, which also indicates the presence of free antenna components. Circular dichroism spectroscopy showed that moderate salt stress had a significant effect on the macro-organization of protein complexes and severe salt stress was further accompanied by changes in the composition of the pigment-protein complexes. No significant differences were observed between the behaviour of *pgrl1* mutant and the WT; on the other hand, in *stt7* cells the PSII fluorescence was less affected in moderate salinity medium; however, at high salinity presence of more detached LHCs was indicated.

In summary, our results evidence that redistribution of excitation energy between PSII and PSI, similar to state 1–state 2 transition, is involved in the protection of cells against moderate salinity. At higher salt concentrations, a general disorganization of the photosynthetic membrane leading to uncoupling of pigment-protein complexes is the most prominent effect.

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Poster

Ameliorating effect of nitric oxide on cadmium stress induced photosynthetic damage in pea (*Pisum sativum* L.)

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Cadmium (Cd), is a life threatening hazardous heavy metal abundant in nature. Cd aggregates to a more elevated amount in leaves than other parts of plants. It primarily affects the photosynthetic apparatus. The primary Cd sensitive sites of the photosynthetic electron transport chain are the oxygen-evolving complex (OEC), NADP oxidoreductase and ATP-synthase. Nitric oxide (NO), a free radical in living organisms, is recently considered as a key signaling molecule in plants. It plays important role in various physiological processes of plants including germination, growth, senescence, photosynthesis and response mechanisms to specific environmental stresses. The present study, the possible ameliorating effect of NO on the overall photosynthesis, starting from PSII to net photosynthesis through PSI, in pea seedlings grown under Cd stress is elucidated.

The pea seedlings were grown hydroponically under cadmium stress conditions (50 and 200 μM $CdCl_2$). Sodium nitroprusside (50 μM) was used as nitric oxide (NO) donor.

Results showed that chlorophyll, Net photosynthetic rate, transpiration rate, stomatal conductance, photochemical efficiency of PSII and PSI decreased, and Fo and non-photochemical parameters for PSII and PSI increased significantly in response to Cd stress, suggesting that Cd affects the efficiency of photochemistry at both PSII and PSI level. Nitric oxide (NO) supplementation through SNP ameliorated Cd stress by enhancing the chlorophyll content, Net photosynthetic rate, transpiration rate, stomatal conductance, photochemical efficiency of PSII and PSI, and reduction in the Fo, non-photochemical parameters of PSII and PSI in pea plants. These data suggested that the exogenous NO application is useful in mitigating the cadmium induced damage to photosynthesis in pea seedling.

Poster

Spectral characteristics of carotenoids and carotenoid containing proteins under exposure to ionizing radiation and singlet oxygen

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To protect photosynthetic apparatus from intense solar radiation cyanobacteria developed water-soluble photoactive Orange Carotenoid Protein (OCP), which contains carotenoid molecule 3'-hydroxyechinenone (hECN). It is responsible for non-photochemical quenching (NPQ) of fluorescence of light harvesting antenna. OCP converts in to quenching red form under blue-green illumination. In this work, we show that OCP may be converted in to the red form under exposure to ionizing radiation.

It was found that molecules of hECN in OCP are bleached by ionizing radiation. Similar effects were observed in experiments where photosensitizer (aluminum phthalocyanine, AlPC [1]) produced singlet oxygen in solution of OCP. However besides overall decrease of carotenoid concentration upon bleaching we observed an increase of optical density at 550 nm indicating formation of the red form of the OCP. Our observations reveal that oxidative stress may also trigger conversion of OCP in to the red form which may also happen *in vivo*, resulting activation of protective mechanisms. Probably such activation of OCP in the dark may be useful to prevent possible effects of high light stress.

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Poster

Performance and thermostability of photosystem II in European beech (*Fagus sylvatica* L.): acclimation rather than adaptation

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Assisted migration of resistant reproductive material may be key in mitigating the effects of climate change on productivity and composition of forest ecosystems. These efforts require understanding of the intraspecific variability in the photosynthetic response of trees to extreme weather events, such as heat waves. In this study we assessed geographical patterns of PSII performance and thermostability of European beech (*Fagus sylvatica* L.), and whether the intraspecific differences can be associated with the climate of origin. Two separate genotypic and phenotypic effects on photosynthetic performance, beech populations growing in two provenance trials with rather contrasting climates were used for the study: Tále (a colder plot, 18 provenances included) and Zbraslav (a warmer plot, subset of 10 provenances). Leaves were sampled both before and after severe heat stress exposure. The fast chlorophyll fluorescence kinetics was used to evaluate PSII performance and the PSII thermostability after simulated heat stress. At non-stressed temperature, the performance of PSII was generally better at the warmer location. The populations close to the Slovenian refugium, as well as closer to the site of origin, showed better performance of PSII, especially after a natural heat-stress event. The effect of simulated heat stress was much stronger at Tále compared to Zbraslav, but only for previously stressed trees. Likewise, we found geographical patterns of thermotolerance indicators as well as relationship between thermotolerance and climate of origin only for stressed trees. While the origin of provenances partly explained the variation among provenances, acclimation driven by the climate plays a major role in response to heat. PSII of beech seems to have a potential to cope with high temperatures.

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Poster

Sensitivity of primary photochemical reactions of sweet sorghum plants to salt stress

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Salinization of soils represents one of the largest environmental challenges worldwide. Since the sweet sorghum [*Sorghum bicolor* var. *saccharatum* (L.) Moench.] is one of the most popular crop used for bioenergetically purpose the study of its sensitivity to salinity stress is very important and actual. In the last decade, genotypic differences in growth rate and biomass accumulation, as well as in efficiency of selected physiological processes have been described in response of sorghum plants to salinity. But, however, exists little information about vulnerability of sorghum PSII photochemistry in relations to physiological mechanism of salt tolerance. In our study, we tested the effect of salinity (0, 100, 150, 200 and 250 mM NaCl) on primary photochemical reactions (using JIP-test) of six sweet sorghum genotypes. The both, increase of Na^+/K^+ ratio as well reduction of plant leaf area after NaCl treatments were used for calculation of two salinity tolerance components: Na^+ extrusion and osmotic tolerance, respectively. The increase in salinity significantly induced the accumulation of the proline and the decline of leaf osmotic potential. Expect for 100 mM NaCl concentration, salinity significantly decreased the leaf chlorophyll content and F_v/F_m. Increasing salinity led to higher accumulation of Q_B-non-reducing PSII reaction centers. Moreover, the Biolyzer software has been used for analysis the salinity effect on the parameters of energy fluxes within leaf. A remarkable finding was observation the K-step occurrence on JIP fluorescence transient in the most sensitive genotypes under high NaCl concentration. Finally, has been observed that the donor side of PSII is more affected by high salt concentration compared to the acceptor side of PSII in sensitive genotypes. Observed down-regulation the primary photochemistry in salt sensitive genotypes resulted from ineffective Na^+ extrusion and raised the ionic imbalance. Results are supported by the image-based analysis of leaf area coloring and more sensitive genotypes showed more significant reduction of green biomass with larger increase of portion chlorotic and necrotic areas of leaves. Importantly, 150 mM NaCl concentration is effective for distinguish of salinity tolerance in sorghum plants. In conclusion, this study provides a view into tolerance mechanisms of different sweet sorghum genotypes to increasing salinity stress and showed a potential for use of sweet sorghum for soil remediation and bioenergetic purposes.

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Poster

LHCSR1 stimulates Photosystem I dependent energy quenching in *Chlamydomonas reinhardtii*

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For photosynthetic organisms, light is essential for their growth. However, when they are exposed to light that surpasses their photosynthesis capability, the excess energy causes harmful reactive oxygen species followed by Photosystem II (PSII) destruction. Thus, they have developed mechanisms to cope with such adverse environments, qE quenching that dissipates excess light energy as heat. In *C. reinhardtii*, LHCSR1 and -3 proteins are involved in this function. According to the previous reports, LHCSR3 contributes to excess light energy dissipation in PSII and the LHCSR3 deficient mutant *npq4* cannot survive under high light conditions. Therefore, this protein has been recognized as a main factor to conduct qE in *C. reinhardtii*. On the other hand, only few things about LHCSR1 function have been known. For further understanding of qE, we performed biochemical and spectroscopic analysis, and revealed that LHCSR1 dependent qE quenching involves PSI. Our results suggest that, energy transfer from LHCII to PSI mediated by LHCSR1 may contribute PSII protection under high light.

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Poster

Ameliorating effects of Mycorrhizae and PGPR on various photosynthetic parameters under Aluminium stress in groundnut (*Arachis hypogaea* L.) seedlings.

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Aluminium (Al) toxicity is one of the primary constraints for plant growth, development and yield in acid soils worldwide. Photosynthesis is the primary process which is affected by Al toxicity.

The objective of this study is to analyze the effects of soil acidity induced Al toxicity with or without the co-inoculation of mycorrhizae and PGPR on different parameters of photosynthesis in groundnut (*Arachis hypogea* L.).

The groundnut (*Arachis hypogea* L. cv. Girinar-3) seeds were grown in plastic pots filled with vermiculite irrigated regularly with or without aqueous solution of $AlCl_3$ (0, 50, 250, 500 and 1000µM at pH 4.5) in combination with mycorrhizal strain, *Glomus etunicatum* and *Pseudomonas putida* species as a PGPR. The effects of these treatments were studied on 25 day old seedlings. The chlorophyll content and Chl fluorescence parameters *viz* quantum yield of photosynthesis (ΦII), electron transport rate (ETR), quantum efficiency of photosynthesis (qP) and non photochemical quenching (NPQ) were analysed as described by Misra et al. (2012) and Kalaji et al. (2014).

A significant decrease in chlorophyll content and photochemical parameters like the quantum yield of photosynthesis (ΦII), electron transport rate (ETR), quantum efficiency of photosynthesis (qP) was recorded in groundnut seedlings with an increase in Al concentration. Co-inoculation of mycorrhizae and PGPR ameliorated Al toxicity induced decrease in the Chl content and efficiency of photochemical parameters.

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Poster

Effect of salt stress on photosynthetic pigments and chloroplasts photochemical efficiency of wheat varieties

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Soil salinity is one of the main extreme environmental factors affecting physiological processes of plants. As soil salinization has recently acquired a global character, modern researches pay much attention to problems related to salt tolerance. In spite of the fact that wheat is not considered to be a salt tolerant plant, gradual salinization of arable soils and the world food problem make necessary the cultivation of wheat in weakly salinized areas. Therefore, the study of the salt tolerance mechanisms, choosing salt tolerant genotypes, their cultivation and use in the breeding are actual problems of the modern biology. To determine salt tolerance of wheat plant, effect of different concentrations of NaCl (150 and 250 mM) on some physiological prosesses occurring in wheat genotypes of *Triticum aestivum* L. (Giymatli2/17, Nurlu99 andAzamatli95) and *Triticum durum* Desf. (Garagylchyg2 vəBarakatli95) has been studied. Stress caused by salinity was shown to influence differently on quantity of photosynthetic pigments (chlorophyll *a*, *b* and carotinoids) in plant leaves depending on its duration. The amount of the pigments increased till the 5th day of the stress and then it began to decrease. It was found that 150 mM concentration of NaCl did not effect significantly on photochemical activities (yield parameter ($F_m - F_0/F_m$) of chloroplasts determined by photosystem II fluorescence. Whereas 250 mM concentration of NaCl led to a significant decrease in the photochemical efficiency of chloroplasts. This decrease was more pronounced in the Garagylchyg-2 genotype as considered sensitive to salinity.

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Poster

Seasonal variations amplify physiological bottlenecks of green algae RILMA1 affecting photosynthetic productivity

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Algae to oil has been explored as a sustainable alternate to fossil fuels whereby industrial scale production can be achieved in a non-intrusive, environment friendly approach when algal cultivation is achieved in marine environments on arid lands. Nevertheless, cultivation of algae is regulated by several intrinsic and extrinsic factors affecting growth, of which tropical seasonal variations are critical. Productivity of industrial algae strain RILMA1 is hampered during winter seasons in open pond cultivation systems due to cumulative light and temperature drop resulting in a less-productive cell physiology. To identify productivity bottlenecks, we made complete physiological profiling of the cells, analysed primary metabolic pathways and ultra-structure of industrial strain RILMA1 exposed to summer and winter conditions in environmental PBR (simulated conditions). Metabolomic profiling and biochemical analysis of winter simulated cells show distinct changes in primary metabolic pathways. Further we observed morphological changes affecting spatial organisation of photosynthetic machinery. This resulted in low productivity. These system wide metabolic, biochemical, and cellular bottlenecks provide cues for cultivation and genetic modifications those will enhance seasonal biomass productivities.

Poster

Extremely high salinity tolerance in *Pongamia pinnata* (L.) pierre, a potential biofuel tree species: Physiological and molecular insights

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Salinity is one of the major environmental constraint limiting plant growth and productivity. Salinity stress has become a serious problem in many regions especially in arid or semi-arid areas. In the present study, we analyzed the extremely high salinity tolerance in *Pongamia pinnata,* a promising biofuel tree species with an insight into the underlying physiological and molecular responses. Our data showed that even at 500 mM NaCl for 15 days, *Pongamia* displayed no deleterious physiological symptoms. Na^+ localization analysis using CoroNa-Green AM revealed effective Na^+ sequestration in the roots when compared to leaves. Elemental analysis demonstrated that roots accumulated more of Na^+ while K^+ content was higher in leaves. At the molecular level, salt stress significantly induced the expression levels of salt overly sensitivel (SOS1), SOS2, SOS3, high affinity K^+transporter (HKT1), ABA biosynthetic and receptor genes (NCED and PYL4), guaiacol peroxidase (POD) exclusively in roots while tonoplast localized Na^+/H^+ exchanger (NHX1) was significantly enhanced in leaves. Our results clearly demonstrate that leaves and roots of Pongamia exhibit differential responses under salt stress although roots are more efficient in sequestering the Na^+ ions. The present study provides crucial inputs for understanding salt tolerance in a tree species which can be further utilized for developing salt tolerance in higher plants.

Poster

Elucidating enhanced photosynthetic efficacy of Maize (*Zea mays*) plants with Arbuscular Mycorrhizal Fungi (AMF) under high temperature stress

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High temperature stress is considered as one of the major destructive stress among various abiotic stresses. This stress has negative implications on plant's morphological, physiological and biochemical growth which ultimately lead to decreased plant productivity and crop yield. Several major soil microorganisms are engaged in mutual symbiosis where arbuscular mycorrhiza fungi (AMF) are the prominent group that forms symbiotic association with more than 80% terrestrial plants. Experiments were performed to elucidate the effects of temperature stress in hot summer days in maize plants colonized with and without arbuscular mycorrhizal fungi (AMF). Various growth parameters (such as leaf number, plant height etc.), chlorophyll (Chl) *a* fluorescence, gas exchange measurement and Chl content were studied in AMF (+) and AMF (–) maize plants during hot summer days. Basic morphological parameters like leaf width, plant height and cob number increased in AMF (+) plants as compared to AMF (–) plants. The results revealed that reaction centers, quantum efficiency of photosystem II (PSII), linear electron transport, energy trapping, performance index, net photosynthesis increased in AMF (+) plants under temperature stress. Total Chl content increased in AMF (+) plants as compared to AMF (–) maize plants. All these results indicated that photosynthesis performance was enhanced in AMF (+) plants as compared to AMF (–) maize plants. This increase indicated that AMF symbiosis helped the plants to cope up with high temperature stress and increased photosynthesis. Among the environmentally safe sustainable endeavours, the alliance of AMF with plant roots was explored and it improved plant growth, photosynthesis and productivity under normal and stressful environment.

Poster

Improved photosynthetic characters correlated with higher biomass in heterotic F_1 hybrid of maize (*Zea mays* L.)

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Heterosis has been exploited in breeding programmes of several plants for developing hybrids that are more vigorous with increased yields than the parental lines. The physiological mechanisms underlying heterosis are not completely understood. In the present study, the photosynthetic efficiency of a heterotic F_1 hybrid (DHM117) that exhibited higher growth rate and biomass was compared with its parental inbreds at vegetative and reproductive stages in the field. The plant height, leaf area and root growth of F_1 hybrid were significantly higher than the parental inbreds at both the stages studied. The net photosynthetic rates (P_n), stomatal conductance (g_s), transpiration rate (E) as well as foliar carbohydrate were higher in F_1 hybrid and parental inbreds at vegetative and reproductive stages. An increase in total chlorophyll content with better chlorophyll *a* fluorescence characteristics including quantum yield (F_v/F_m), photochemical quenching (q_p) and decreased non-photochemical quenching (NPQ) was observed in F_1 hybrid than the parental inbreds. Thus improved photosynthetic efficiency including foliar carbohydrates and chlorophyll content might have contributed to higher growth rate and biomass in F_1 hybrid.

Poster

The influence of the nitrogen source on photochemistry and antenna size of the photosystems in marine green macroalgae, *Ulva lactuca*.

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Ulva lactuca, a fast growing marine macroalgae is regarded as a prospective energy crop for biorefinery. In fast growing macroalgae, the growth and biomass development depends strictly on nitrogen abundance. Additionally, the macroalgal nutrient bioremediation rates and photosynthetic pigment content vary with type of nitrogen source. However, the influence of nitrogen source on photosynthesis is not widely studied in marine macroalgae. In present study, the effect of dissolved inorganic (DIN) and organic nitrogen (DON) in *U. lactuca* is determined through *in vivo* chlorophyll (Chl) *a* fluorescence kinetics. The PSI and PSII antenna size heterogeneity in relation to variation in nitrogen source is analysed. A detailed analysis of the $P700^+$ re-reduction kinetics using diuron alone and combination of inhibitors is studied. Further, PSI photoprotection under high light conditions has been assessed by studying the intersystem electron transport chain (ETC).

Poster

Ferredoxin: The Central Hub Connecting Photosystem I to Cellular Metabolism

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Ferredoxin (Fd) is a small soluble iron-sulfur protein that is ubiquitous and essential in all oxygenic photosynthetic organisms. It is considered one of the simplest polymetallic proteins with a single [2Fe-2S] cluster, composed of two iron atoms bridged by two sulfide atoms and coordinated by four cysteine ligands. It accepts a single electron form the stromal surface of Photosystem I (PSI) and shuttles the electron to a wide range of electron acceptors that are involved in multiple and diverse metabolic processes. This includes generation of NADPH via Fd-NADP-reductase for carbon dioxide fixation, cyclic electron transport for ATP synthesis via cytochrome b_6f complex, nitrate reduction via nitrite reductase, sulfite reduction, production of hydrogen via [2Fe-2S]-hydrogenase and many other reductive reactions. It functions as the central hub for many cellular redox reactions and is integrated into a large network in the cellular metabolism. Here we present recent advances to highlight this central importance of Fd and its evolutionary significance from cyanobacteria to algae and higher plants. We compare the expression and structural diversity of different Fd gene products to understand the central role of Fd in these organisms. This will shed light on the mode of regulation and specificity for how Fd partitions reducing potential between competing partner proteins to enable the maximum contribution of PSI to the cell physiology and metabolism.

Poster

Growth and photosynthesis in *Jatropha curcas L.* in response to water and salinity stresses

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Utilization of non-arable land for growing biofuel feedstock will unriddle the scuffle for land usage between food crops and biofuel trees. *Jatropha curcas* is a biofuel tree species which is known for its high oil content, abiotic stress tolerance and phytoremediation. In the current study, we established its potency for abiotic stress tolerance including interactive drought and salt stress with emphasis on its growth and photosynthetic characteristics. *J. curcas* plants were treated for 7, 15, 21 days with individual and interactive drought and salt stress by continuous water withholding and 200mM NaCl treatment. With progression of stress there was a decrease in leaf relative water content, stem height and width, number of nodes and total fresh weight of the plant. Gas exchange parameters including photosynthetic rates (P_n), stomatal conductance (g_s), transpiration rates (E) and apparent quantum efficiency were significantly decreased under stress treatments. This decrease was more pronounced under combined treatment of drought and salt stress when compared to the individual stress treatment. Water use efficiency (WUEi) was retained till 7 days after stress (DAS) but decreased under prolonged stress treatments. Our results on photosystem II efficiency showed decreased quantum yield and photochemical quenching and an increased non-photochemical quenching. Also, Progressive drought and salinity stress induced a gradual accumulation of H_2O_2 and lipid peroxides in *J. curcas.* In conclusion, *J. curcas* demonstrated morphological, physiological and biochemical adaptive mechanisms under progressive drought and salinity stress which suggest that Jatropha can be a potential biofuel crop even under most unfavourable environmental regimes in the changing climate.

Poster

Salinity induced regulations of organic metabolites, ion homeostasis and antioxidative defense maintain the redox status of the cells and the structural integrity of photosynthetic apparatus and contribute in salinity tolerance of the xero-halophyte *Haloxylon salicornicum*

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The halophytes are the viable organisms that are naturally adapted to high saline environment. They have an array of adaptive mechanisms that enable them to adapt to high saline conditions. *Haloxylon salicornicum (*Moq.) Bunge is a xero-halophytic species that can grow efficiently in dry saline areas. The present study investigates the changes in growth, photosynthesis, water use efficiency, chlorophyll fluorescence, mineral nutrient levels, the accumulation of organic metabolites, antioxidative defense system in the xero-halophyte *H. salicornicum* subjected to various levels of salinity (0–400 mM NaCl) for 21 d in order to assess the salinity tolerance mechanism. The seedlings of *H. salicornicum* were survived till the end of the treatments even at high dose of NaCl (400 mM NaCl). Salinity did not induce any significant changes in the levels of K^+, Ca^{2+} and Mg^{2+} in both shoot and root. The fresh and dry biomass of *H. salicornicum* increased in NaCl treated seedlings and shoot water content (SWC %) remained unaffected by salinity. The levels of H_2O_2 increased under salinity, whereas $O_2^{\bullet-}$ level remained unchanged. The lipid peroxidation level and remain unchanged at low salinity and increased under high salinity. Various photosynthetic pigment content remained unaffected up to 300 mM NaCl treatment and it decreased significantly at 400 mM NaCl treatment. The photosynthetic rate (P_N) and Water use efficiency (WUE) decreased in NaCl-treated seedlings as compared to control. The salinity had no significant effects on PSII efficiency as indicated by unchanged levels of F_v/F_m ratio and the photochemical quenching (qP). The ratio of AsA/DHA (indicators of cellular redox potential) was elevated in the seedlings subjected to salinity treatments. The activities of various enzymatic antioxidative components such as SOD, APX, POX and GR increased at all levels of salinity as compared to control. On the other hand, the activity of CAT increased at low dose of salinity and decreased under high salinity. Taken together, our result suggest that efficient coordination between enzymatic and non-enzymatic antioxidants, accumulation of organic metabolites, ion homeostasis, maintenance of water status, minimal pigment degradation and protection of PSII form salinity induced oxidative damage are important factors contributing to the salinity tolerance of *H. salicornicum.*

Poster

Response of cell wall bound β-galactosidase to photosynthetic loss of sugar production during dark induced and natural senescence in *Arabidopsis* leaves

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Leaf senescence in natural environment or induced by continuous darkness monitored in the laboratory conditions causes loss in photosynthesis consequently loss in the level of cellular sugar in the leaves of *Arabidopsis thaliana*. On the other hand, execution of senescence program needs energy and therefore, the senescing cells receive sugars from other sources. Cell wall polysaccharides are the richest source of organic carbon and are broken down by the action of several hydrolases to respiratory sugars. We have demonstrated sugar starvation as a signal for induction of the activity of cell wall bound β-galactosidase, a model enzyme of the hydrolases to investigate the efficiency of its activity during senescence in the background of loss in photosynthetic production of sugars [1, 2]. Not unexpected, the loss of sugar during natural senescence was relatively slow compared to its severe loss during senescence induced by darkness. Importantly, the enzyme activity is found to increase by several folds in dark induced senescence compared to the increase in the leaves senescing in natural light dark environment. The data therefore, indicate that the loss of photosynthetic production of sugar is a modulator of activity of the enzyme that in association with other hydrolases breaks down the wall polysaccharides to sugars to sustain energy homeostasis during senescence. The precise mechanism of action of the enzyme for degradation of cell wall polysaccharides associated with the cell wall is not resolved yet. The experimental data with excised leaves on the enhancement in the activity of the enzyme by photosynthetic inhibitors and suppression of the activity by exogenous sugars suggest possible involvement of a kind of sugar signalling network in the cell wall catabolism during senescence.

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Poster

Proficient modulations of the antioxidative defense system and ion homeostasis maintains the structural and functional integrity of the photosynthetic apparatus and confer drought tolerance in the facultative halophyte *Salvadora persica* L.

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Salvadora persica L. is a facultative halophyte growing in arid and semiarid regions of India. The plant has wide adaptability and it can tolerate high salinity, drought and water logging conditions. In the present study, the seedlings of *Salvadora persica* L. were imposed to drought stress by withholding the irrigation for 15 days and recovered from drought after re-irrigation, for assessing the drought tolerance mechanisms in *S. persica*. Various growth parameters, mineral nutrient contents, ROS levels, variations in antioxidative enzymes, photosynthetic parameters, chlorophyll fluorescence were measured under drought and recovery conditions. Our results showed that there was a reduction in fresh as well as dry biomass, leaf area and relative water content (RWC %) in the plants imposed to drought stress as compared to control plants. While, upon re-irrigation the drought treated plants resumed their growth at par to the control level. The photosynthetic pigment content, net photosynthetic rate (P_N), intercellular carbon dioxide concentration (C_i), stomatal conductance (g_s) and transpiration rate also declined under water deficit stress and increased during recovery phase. However, the maximum quantum efficiency of PS II (F_v/F_m), photochemical quenching (qP), non-photochemical quenching (NPQ), electron transport rate (ETR) and quantum yield of PSII (Φ PS II) remained unchanged during the water deficit condition which indicate that the integrity of photosystem II is maintained under drought stress condition. The reduction in P_N was due to stomatal limitations on photosynthesis. The analysis of antioxidative enzymes showed that the activity of catalase decreased, whereas the activity of guaiacol peroxidase (POX) increased under drought stress. However, the activities of superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR) remained unaffected during water deficit condition. The ionomics studies revealed that the levels of Na^+, Ca^{2+}, Mg^{2+} and Mn^{2+} increased and K^+ content remained steady during drought and this results indicate that these ions may contribute in maintenance of charge balance, structures of electron carriers and the enzyme activities. Our data strongly propose that the reduction in growth, efficient regulations of antioxidative enzymes and ion homeostasis are the major contributor in maintaining the structural and functional integrity of photosynthesis apparatus and thereby accomplishing the drought tolerance in *S. persica*.

Poster

Uncovering bottlenecks in photosynthesis for microalgal biomass production in changing environment for bioenergy

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The rising demand for renewable energy sources and vegetarian protein rich feed across the globe put algae as a disruptive agricultural crop. Algae are fast growers, their cultivation is scalable and sustainable because they can grow in salt water and with immense contribution to CO_2 mitigation. Fuels produced from algae have the potential as jet fuel and can replace conventional energy resources. The sustainable production of algal biofuel and biomass depends on the year round cultivation of algae. However abiotic stress impedes the functions of the photosynthetic process and consequently their proliferation. It is thus desirable to identify the bottlenecks in the kinetics of the light reactions of photosynthesis and devise strategies to overcome those for sustaining outdoor productivities round the year. With this motivation RILMA1 (Reliance Industries Ltd. MA1) industrial strain was evaluated under seasonal variations for photosynthetic performance. The strain was grown in environmental PBRs (ePBR, mimicking outdoor raceway ponds) under summer and winter conditions. The impact of the growth light and temperature on the photosynthetic performance was recorded as oxygen evolution based Photosynthesis-Irradiance (P-I) curve and Chlorophyll *a* fluorescence kinetics. Our experimental results revealed that photosynthetic performance of RILMA1 strain varies with seasonal fluctuations in light and temperatures. These results uncovered the bottlenecks in primary photochemical reactions of photosynthesis. This could be exploited in large scale microalgal mass cultivation for enhancing productivities through process engineering and genetic modifications.

Poster

Drought-induces changes in physiological processes and recovery of spruce seedlings from contrasting provenances

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In July 2013 was conducted pot experiment with 8-months spruce seedlings originated from two Slovak provenances (PV1 – 410 a. s. l.; PV2 – 931 a. s. l.). The aim of the study was to identify physiological response of these seedlings in conditions of drought and subsequent rewatering. Experiment was conducted in climate room with controlled mode of moisture, temperature and light conditions. Seedlings from each provenance were arranged in two variants: drought (S) and control (K). Seedlings of variant control were regularly watered in 3-days intervals, variant drought was grown for 8 days without watering. Then they were re-irrigated to track the *"recovery"* process. Effect of dehydration and re-irrigation was monitored at the level of water potential (Ψ_w), net photosynthetic rate (P_N), stomatal conductance (g_s), accumulation of proline and abscisic acid (ABA).

8-days of limited watering caused decrease in values of Ψ_w to -1,14 MPa (PV1), -1,07 (PV2). Progressive dehydration caused inhibition of P_N, g_s and induced significant accumulation of proline. We recorded significantly increased values ABA concentration in needles (PV1 – 7,8 times; PV2 – 5,3 times) at the end of phase without watering. Provenance PV1 originated from wetter climate region responded to water deficit more sensitive than provenance PV2 originated from dryer climate region. Rewatering of seedlings in drought treatment caused restoring of values all measured parameters to values level recorded in the initial measurements. We recorded the quickest recovery of physiological functions by provenance PV1 originated from the lower limit of spruce extension.

Poster

Drought tolerance is highly coordinated with photosynthetic efficiency and leaf water relations in two mulberry (*Morus* spp.) genotypes under progressive drought stress

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The present study was aimed to investigate the drought stress responses on photosynthetic physiology in two mulberry (*Morus* spp.) genotypes including a drought tolerant (DT) Selection-13 (S13) and a drought susceptible (DS) Kanva-2 (K2). One year old mulberry genotypes of S13 and K2 were subjected to natural drought stress for 10 and 20 days (D10 and D20, respectively). Progressive drought stress(PDS) caused significant reduction in net photosynthetic rates (P_n), stomatal conductance (g_s), leaf hydraulic conductance (K_L), midday water potential (Ψ_{md}), chlorophyll a fluorescence, and performance index (PI_{ABS})) characteristics compared to their respective controls. Among the two genotypes, S13 showed significantly higher rates of P_n, instant water use efficiency, K_L, Ψ_{md} and higher chlorophyll a fluorescence characteristics suggesting a better Photosystem-II (PS-II) efficiency compared to K2 under PDS. Leaf hydraulic dynamics were highly coordinated with better photosynthetic rates as well as Photosystem-II efficiency resulting in superior growth and biomass even under PDS. Our data clearly suggest that the leaf water relations play a key role in photosynthetic carbon assimilation patterns and PS-II efficiency during PDS which could be effectively targeted towards mulberry improvement programs for drought adaptation in the future changing climate scenario.

Poster

Novel Cu(II)-organic inhibitors of carbonic anhydrase, glutation reductase and photosynthetic activity of plant photostystem II

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Design of novel compounds effectively suppressing several metabolic processes, and therefore capable of achieving the synergism effect would serve as the perspective approach to weed management and one of the ways to increase the efficiency of plant growth regulation. On the other hand, highly specific inhibitors can be useful as a sophisticated instrument in scientific studies. Copper cations are known to be the one of essential micronutrients for plant growth. As a significant cofactor of many important enzymes copper (Cu) plays an important role in numerous metabolic processes in all photosynthetic organisms (i.e., cyanobacteria, algae and plants). On the other hand, free Cu ions induce oxidative damage of cells catalyzing the formation of reactive oxygen species. It was revealed that the components of photosystem II (PSII) are the most sensitive to inhibitory effect of Cu. Organic ligands of chelate complexes with metal cations significantly increase the availability of the biosystem regions sensitive to the metal effect; thus, the inhibitory activity of such complexes is increased as well. Copper complexes with organic ligands better inhibit photosynthesis and other cellular reactions. It was shown that complexes based on the copper salts and some organic derivatives can inhibit several biological activities. A series of nine novel Cu(II) complexes and four ligands was evaluated as inhibitors of photosynthetic electron transfer in spinach thylakoids (*Spinacia oleracea* L.). *In vitro* inhibitory potency of these agents against: photochemistry and carbonic anhydrase activity of photosystem II (PSII); α-carbonic anhydrase from bovine erythrocytes; as well as glutathione reductase from chloroplast and baker's yeast (*Saccharomyces cerevisiae*) were studied. It was shown that all Cu(II) complexes exhibited excellent inhibitory effect on both glutathione reductase and α-carbonic anhydrase activity. Some of them also performed good inhibition of the photosynthetic as well as carbonic anhydrase activity of PSII. The ligands were shown to have inhibitory effect lower than that of the Cu(II) complexes, but nevertheless the activity does not depend on copper content. As these compounds were synthesized for the first time, the mechanisms and features of their action require detailed investigation. However, this study allows finding of such compounds that will help people to solve urgent agricultural and medical problems in the future.

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Poster

Effects of H_2O_2 and SNP (nitric oxide donor) on antioxidant defense mechanisms in leaf discs of pea, *Pisum sativum*

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Reactive oxygen species and nitric oxide (NO) are produced routinely during various metabolic pathways that are localized in different cellular compartments such as chloroplasts, mitochondria and peroxisomes. We attempted to study the effects of exogenous hydrogen peroxide (H_2O_2) and sodium nitroprusside (SNP, a NO donor) on leaf discs of pea, *Pisum sativum* in dark, moderate (300 μmol m^{-2} s^{-1}) or high light (1200 μmol m^{-2} s^{-1}). The reactive oxygen species levels (H_2O_2 and O_2^-), antioxidant enzyme activities, total chlorophyll and carotenoid contents were examined under treatment with external hydrogen peroxide (H_2O_2) and SNP. External hydrogen peroxide (0.1 mM) lead to the accumulation of superoxide in moderate or high light. The treatment with 2.5 mM SNP resulted in accumulation of high levels of superoxide in moderate light as compared to dark but in case of high light superoxide levels were decreased. This may be because of degradation of hydrogen peroxide and sodium nitroprusside in high light. During H_2O_2 treatment, the total chlorophyll content decreased in high light but only marginal or no effect on the carotenoid content. Treatment with SNP lead a moderate increase in total chlorophyll and carotenoid content in leaf discs exposed to high light followed by moderate and dark. This means that SNP may also plays a role in increasing the chlorophyll and carotenoid content. Experiments are underway to examine the patterns of phosphoenolpyruvate carboxylase, glycolate oxidase, catalase on exposure to H_2O_2 or SNP.

Poster

Interactive effects of CO_2 enrichment and drought stress on photosynthesis and antioxidant machinery in short rotation coppice (SRC) mulberry, a potential bio-energy tree species

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Present study investigated the interactive effects of elevated [CO_2] (550 µmol mol^{-1}) and drought stress (DS), which are two important global climate change factors, on photosynthesis and antioxidant machinery in short rotation coppice (SRC) mulberry, a potential bio-energy tree. Elevated [CO_2] (E) stimulated photosynthetic performance in well watered (WW) as well as during DS with significant increases in light saturated photosynthetic rates (A_{Sat}), intercellular [CO_2], water use efficiency (WUE_i) and photosystem II efficacy (F_v/F_m and $\Delta F/F_m'$) with respect to ambient [CO_2]counterparts. Diminished levels of H_2O_2, proline and malondialdehyde as well as increased contents of antioxidants including ascorbic acid and total phenolics in WW as well as during DS conditions in high CO_2 grown plants indicated lesser oxidative damage. Further, ambient [CO_2] grown plants showed higher transcript abundance and antioxidant enzyme activities under WW as well as during initial stages of DS (15 days). Nevertheless, with the increasing of DS imposition (30 days), ambient [CO_2] plants showed down regulation of antioxidant systems compared to their elevated [CO_2] grown counterparts. Our data clearly demonstrated that future increased atmospheric [CO_2] enhances the photosynthetic potential and also mitigates the drought- induced oxidative damage in SRC mulberry.

Poster

Gas-exchange measurements, fluorescence and complete protein profile of barley plants against salinity and UV-B, singly and in combination

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Responses of barley to single and combined effects of salinity and UV-B were assessed. In the present study, 21 days old barley plants were exposed to UV-B (+7.2 kJ m^{-2} d^{-1}) and salinity (100 mM NaCl), singly and in combination. Both the stresses singly affected various physiological processes, for instance, salinity limited the RuBP regeneration and checked triose phosphate utilization while UV-B affected RuBisCo carboxylation efficiency. The two stresses in combination increased the mesophyll conductance maximally and increased the Ci. Various physiological changes led to decline in carbon assimilation with maximum in UV-B, followed by UV-B + salinity and salinity. Complete protein profile was obtained under different treatments and differential expressions were compared and it was found that the number of protein expressed reduced under different treatments compared to control. Under salinity, UV-B and salinity + UV-B, different proteins related with protection and repair were expressed. Inductions in different antioxidative enzymes were obtained under different treatments, singly and in combination with maximum being under UV-B+salinity except GR which was induced only under salinity stress. Salinity stress induced osmolytes and thus may have provided protection to plants against osmotic/ionic stress. Among salinity and UV-B, UV-B affected plants survival and productivity the most. Combined stress led to less than additive effects.

Poster

Evidence of seed photosynthesis and its role on storage product accumulation in the developing green seeds of *Pongamia pinnata* (L.) Pierre, a potential biofuel tree species

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Seed development is an intriguing phenomenon with a complex network of intricate machinery control by both maternal and filial tissues. In addition to sucrose supply from the maternal side, seed itself can provide a carbon source which plays a crucial role in synthesizing its major storage reserves. The synthesis of major storage reserves as lipids is an energy expensive process. Green seeds contain photosynthetically active plastids referred as the photoheterotrophic plastids. In presence of light, these plastids provide ATP and NADPH as the energy supply for lipid biosynthesis releasing O_2 for mitochondrial respiration. *Pongamia pinnata* is a legume tree species known to be an efficient protein and oil accumulator. About 30–40% of seed biomass is lipids and 50–60% of the total lipid content is oleic acid. In this study. we recorded a suitable correlation with light availability and storage product synthesis in the developing green seeds of *Pongamia pinnata*. Biochemical analysis at four major developmental stages: 140 DAA, 200 DAA, 260 DAA and 320 DAA. There was a significant increase in total chlorophyll content from 140 DAA to 200 DAA. Further, the expression of major photosynthesis related genes in different developing stages were analysed. In order to check the effect of light on storage metabolites, seeds were allowed to develop either under natural light or under dark-incubation without providing any barrier between the other parts of the tree. Interestingly, the lipid content decreased in dark incubated seeds with no significant difference in protein or starch accumulation. Moreover, the dark incubated seeds showed a significant reduction in seed dry weight and increase in relative seed moisture content. Our data demonstrate the crucial role of light in regulating carbon economy and regulation of lipid biosynthesis during seed development in Pongamia.

Poster

Modulation of photosynthesis associated with phytostabilization of zinc in a halophyte – *Acanthus ilicifolius* L.

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The ever increasing problem of environmental pollution by toxic heavy metal ions can be effectively tackled with the help of green plants. *Acanthus ilicifolius* L. was recently proved to be a suitable candidate for phytostabilization of zinc (Zn) (Shackira *et al*., 2017). The present study was designed to investigate the photosynthetic responses of *A. ilicifolius* plants during the increased accumulation and stabilization of Zn inside the plant tissue. Hydroponically grown (half strength Hoagland medium) stem cuttings of *A. ilicifolius* with a pre-growth period of 35 d were treated with 4 mM $ZnSO_4$ for a period of 15 d. Changes in the Chl *a* fluorescence parameters, PSII activity and stomatal parameters with regard to the increased accumulation of Zn in the root tissue was studied in detail. The JIP test proved that the control plants exhibited a polyphasic rise in the OJIP transient while the $ZnSO_4$ treated samples recorded significant reduction. Similarly, the $ZnSO_4$ treatment led to a decline in the number of active reaction centres of PSII and rate of electron transport as revealed by the energy pipeline models derived from the Chl *a* fluorescence analysis. This decrease was in correlation with the decreased PSII activity of $ZnSO_4$ treated samples as compared to the control leaves. In addition, SEM images revealed that the stomata were partially closed in the leaves of $ZnSO_4$ treated samples as compared to the fully opened stomata of control leaves. Even though a reduction in photosynthesis was observed, the antioxidant system seems to be highly active as increased accumulation of ascorbate and glutathione content were observed in the leaf tissue. Thus, the photosynthetic reduction of *A. ilicifolius* plants treated with $ZnSO_4$ might be a metabolic adjustment of the plant to overcome the phytotoxicity of Zn by diverting the growth processes to maintenance processes during the phytostabilization of the toxic metal.

1. Shackira AM, Nabeesa Salim E, Jos T Puthur. (2017). *Acanthus ilicifolius* L. – a promising candidate for phytostabilization of zinc. Environ. Monit. Assess. 189(282), pp. 1-13

Poster

Heat-Induced Programmed cell death is mediated by an Ssl2245-Sll1130 Toxin-Antitoxin system in the cyanobacterium, *Synechocystis* sp. PCC 6803

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Two putative heat responsive genes, *ssl2245* and *sll1130* constitute an operon that also has characteristics of a toxin-antitoxin system, thus joining several enigmatic features. Closely related orthologs of Ssl2245 and Sll1130 exist in widely different bacteria, which thrive under environments with large fluctuations in temperature and salinity, among which some are thermo-epilithic biofilm forming cyanobacteria. Transcriptome analyses revealed that the CRISPR genes as well as several hypothetical genes were commonly up-regulated in Δ*ssl2245* and Δ*sll1130* mutants. Genes coding for heat shock proteins and pilins were also induced in Δ*sll1130*. We observed that the majority of cells in a Δ*sll1130* mutant strain remained unicellular and viable after prolonged incubation at high temperature, 50°C. In contrast the wild type formed large cell clumps of dead and live cells, indicating the attempt to form biofilms under harsh conditions. Further, we observed that Sll1130 is a heat-stable ribonuclease, whose activity was inhibited by Ssl2245 at optimal temperatures, but not at high temperatures. In addition, we demonstrated that Ssl2245 is physically associated with Sll1130 by electrostatic interactions, thereby inhibiting its activity at optimal growth temperature. This association is lost upon exposure to heat due to changes in conformation of the Sll1130 protein, leaving Sll1130 to exhibit its ribonuclease activity. Thus the activation of Sll1130 leads to the degradation of cellular RNA, there by heat-induced programmed cell death that in turn supports the formation of a more resistant biofilm for the surviving cells. We have identified for the first time, this programmed system leading to death in a part of the population, aiding the survival of the rest till the return of favourable conditions in *Synechocystis* PCC 6803 and suggest to designate Ssl2245 and Sll1130 as MazE and MazF respectively.

Poster

Maintenance of reactive oxygen species, cellular membrane integrity and engagement of C_4 photosynthetic enzymes in four cultivars of onion seedlings under salt stress

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Salinity is a major threat in plant agriculture led to develop salt tolerant crops for sustainable agriculture. The present investigation was carried out to reveal the responses of reactive oxygen species, C_4 photosynthetic enzymes, proline and membrane integrity in seedlings of four selected onion (*Allium cepa* L.) cultivars Agrifound rose (AF), Bellary (B), Prema-178 (P-178) and Nasik red (NR) at randomized time (3, 6, 24 and 48 h) intervals of after treatment with NaCl (0, 50, 100, 150 and 200 mM). The response of the cultivars varied with salt concentration and also by treatment duration. 1-3 fold increase in catalase, glutathione reductase, ascorbate peroxidase and superoxide dismutase was noticed in B, P-178 and NR. Maximum phosphoenol pyruvate carboxylase, NADP dependent malic enzyme and pyruvate orthophosphate dikinase levels were observed in P-178 followed by B after 24 h of salt exposure at 100 and 150 mM NaCl. Malondialdehyde content was less in P-178 compared to other cultivars. Significant increase in proline accumulation was monitored in P-178 and B at 100 mM NaCl after 24 h of salt induction. P-178 cultivar appeared to be more tolerant to salinity than other three cultivars with good antioxidant, proline accumulation, maintaining membrane integrity and high C_4 photosynthetic enzymes involved in acclimation to stress, we find somewhat similar results with B. P-178 cultivar expressed tolerant responses by least accumulation of H_2O_2 in onion seedlings. We suggest that P-178 as indicated by DAB staining compared to other cultivars may further be exploited.

Poster

A photochemical reflecting index as perspective method for remote monitoring of plant photosynthesis under changeable conditions: meta-analysis of literature data

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A photochemical reflectance index (PRI) is calculated as ratio of difference of intensities of reflected light at 531 and 570 nm to sum of these intensities. It is possible that PRI includes two components: the fast component is connected with change in pH gradient across the thylakoid membrane; the slow component is connected with changes in the xanthophyll cycle. Now, many works analyze connection of PRI with important photosynthetic indices including quantum yield of photosystem II (Φ_{PSII}), nonphotochemical quenching (NPQ), efficiency of photosynthetic light energy utilization (LUE), net CO_2 uptake, etc. under stress conditions. Methods of PRI application for monitoring of photosynthesis in agricultural plants under different conditions of environment are developed. Nevertheless there are problems for using of PRI in agricultural investigations. Particularly, the correlation coefficient between photosynthetic indices (Φ_{PSII}, NPQ, LUE, net CO_2 uptake) and PRI varies from 0.1 and lower to 0.95 and more. This variability can be explained by the different contribution of xanthophyll cycle pigments to photoprotection of photosystem II in different plants and by participation of other mechanisms of photosynthetic regulation on different levels. A presence of trichomes and surface irregularities on plant leaves can strongly decrease these correlations (<0.1). The correlations between PRI and photosynthetic indices can depend on part of a day and season, when measurements are performed. Finally, correlations depend on localization of sun, angle of slope of leaves to sensor, reflecting properties of soil, etc. Thus, the problem of PRI application in agricultural plants monitoring requires solution of following tasks: influence of relative position of light source and leaves on PRI should be minimized; models of influence of leaf structure on leaf reflecting properties should be elaborated; mathematical models of photosynthesis efficiency and PRI under stress conditions should be developed; these models should be adapted for monitoring of certain agricultural plant varieties. A making databases, including information about influence of stress conditions on photosynthesis and PRI, and their theoretical analysis will contribute to reveal the most effective design of monitoring of agricultural plants in fields.

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Poster

Study of photochemical activity of PS II and chlorophyll fluorescence emission spectra from corn mesophyll and bundle sheath cells

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Plants with C_4-photosynthetic metabolism have higher productivity and effective biomass gaining ability compared to C_3-plants. The leaves of corn (*Zea mays* L.) absorb CO_2 from the air via C_4 type photosynthesis, and the carbon assimilation process in this case is split in two cycles. The separation of these two cycles inside the leaf is managed by two specialized photosynthetic cells: bundle sheath cells (BS) compactly located around vascular bundles and mesophyll cells (M) surrounding them. The main purpose of the presented work is the comparative study of photochemical activity of photosystem II and chlorophyll fluorescence emission spectra in the assimilative tissues isolated from corn leaves. Zaqatala 420 maize cultivar was used as the object of this study. It was determined that the activity of photosystem II is 167 μmol $O_2 \cdot mg^{-1}$ chlorophyl$\cdot s^{-1}$ in the chloroplast isolated from mesophyll cells and 34 μmol $O_2 \cdot mg^{-1}$ chlorophyl$\cdot s^{-1}$ in the bundle sheath cells. The activity of PS II in bundle sheath cells is approximately 5 times lower than in the mesophyll cells. The spectral properties of chlorophyll of granular and non-granular chloroplasts were studied by the method of low-temperature fluorescence (77 K). The analysis of fluorescence spectra of chlorophyll in maize leaves showed the presence of three maxima, characteristic of light-harvesting complex at 686 nm, a PS II complex at 695 nm, and a PS I complex at 735 nm. It is shown that these three maxima are also present in maize mesophyll chloroplasts. However, in the fluorescence spectrum of coating plastids, there are almost traces of the band at 695 nm. The absence of this fluorescence band pertaining to the chlorophyll-protein complex of PS II is considered as a violation in its antenna chlorophyll. The presence of trace amounts of this band of chlorophyll is most likely due to the preservation of individual contact sites between neighboring thylakoids. In this case, the low level of 695 nm band in the fluorescence spectra is suggested to be associated with a small amount of light-harvesting complex and very low electric transport activity.

Poster

Triplet-cysteine Motif Repeat protein 1 (TMR1): A novel chloroplast protein necessary for accurate control of cyclic electron transfer around photosystem I

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In plant cells, chloroplasts carry out many important functions including photosynthesis, the most crucial reaction of life on the Earth. However, mechanisms of how chloroplast functions are regulated have been largely unknown yet. Triplet-cysteine Motif Repeat protein 1 (TMR1) is a novel nuclear-encoded chloroplast protein that may control chloroplast functions, although its exact function has not been characterized. Here, by using a genome-editing CRISPR/CAS9 system, we isolated the Arabidopsis *tmr1* knock-out mutant line (*tmr1-2*). We also succeeded to isolate the *TMR1* over-expression line (OE8-8) that shows approximately 63-fold higher *TMR1* transcript levels than that in wild type (WT). Although the shoot of *tmr1-2* and OE8-8 plants showed the WT phenotype under green-house conditions, the Pulse Amplitude Modulation (PAM) analysis revealed the significant differences in Non-photochemical quenching (NPQ) values between WT and the mutants. In details, the overexpression of *TMR1* enhanced the NPQ values under high actinic light (AL) condition when the analysis was carried out with plants grown under short-day and high-light-stress condition. Furthermore, chlorophyll fluorescent kinetics of OE8-8 was significantly higher than that of WT after turning off the AL, suggesting that cyclic electron flow from federoxin (Fd) to plastoquinon (PQ) pools was accelerated in the overexpression line. In contrast, NPQ values in *tmr1-2* were lower than those in WT when PAM analysis was performed with plants that were pre-incubated for overnight in the dark. The light curve-based analysis of the PSI acceptor side limitation (Y(NA)) showed that there is no limitation of electron sink at the acceptor side of PSI in *tmr1-2* under high AL intensities; however, the electron sink was increased in OE8-8 than in WT under the same AL conditions. Based on the results obtained, we suggested that TMR1 functions as an electron buffer for controlling cyclic electron transfer during electron sink limitation.

Poster

Characterization of ABC transporter genes, sll1180 and sll1181 involved in acid stress tolerance of *Synechocystis* sp. PCC 6803

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In the *Synechocystis* sp. PCC6803 genome, over 50 ABC transporter-related genes have been detected by genome sequence analysis. To identify ABC transporters involved in acid resistance, deletion mutants of other substrate-unknown ABC transporter genes were screened for their acid-stress sensitivities in a low-pH medium.

A mutant of *sll1180*, which encodes proteins with respective homology to HlyB in *E. coli*, was found to be more sensitive to acid stress than wild-type cells. The abundance of expression of the genes was analyzed under acid stress condition by quantitative real time reverse transcriptase-polymerase chain reaction. The expression of sll1180 increased in the wild-type cells after acid stress treatment. These results suggest that Sll1180 has an important role in the growth of *Synechocystis* sp. PCC6803 under acid stress condition. To reveal the localization of Sll1180, we performed Western blot and immunofluorescence. These results showed that Sll1180 localized in plasma membrane.

Since HlyB, HlyD and TolC complex transport HlyA in *E. coli*, we searched for genes corresponding to them from Synechocystis sp. PCC6803. BlastP search suggests that HlyA, HlyD and TolC proteins has homology to Sll1951, Sll1181 and Slr1270. We constructed deletion mutant of these genes. The Δ*sll1180* and Δ*slr1270* cells showed acid stress sensitivity. The BACTH analysis showed that Sll1180 interacted Sll1181 and Sll1951. Form dot blot analysis of Sll1951-His, the Δ*sll1180* cell did not transport Sll1951-His from cytoplasm to extracellular.

These results suggest that Sll1180 and Sll1181 transport Sll1951 and Sll1951 outside of the cells might be a key factor of acid stress tolerance.

Poster

Effect of elevated CO_2 on growth, yield and seed quality of Pigeonpea

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Atmospheric CO_2 has been increasing continuously due to anthropogenic reasons. The preindustrial CO_2 concentration of 270 µmol mol^{-1} has now reached 400 µmol mol^{-1}. Growth under elevated CO_2 resulted varied responses in photosynthetic metabolism among different crops. Pigeonpea (*Cajanus cajan L.*) is widely cultivated in tropical and subtropical regions and is an important legume crop as a major protein source. The current study was aimed at understanding the effect of elevated CO_2 on Pigeonpea seed yields and nutritional quality. Pigeonpea was grown in open top chambers under elevated (600 µmol mol^{-1}) and ambient CO_2 to assess the yields and seed nutritional quality in terms of C and N status. Our data demonstrated enhanced growth and biomass in Pigeonpea grown under elevated CO_2 during both vegetative and reproductive stages with higher seed yields compared to the ambient controls. There was an increase in seed total carbohydrate and reducing sugar contents under elevated CO_2. However, the sucrose and fructose contents were less in elevated CO_2 compared to ambient plants. Total protein and free amino acid levels were higher in elevated CO_2 grown plants indicating the absence of N limitation in Pigeonpea. Further studies are in progress to understand the biochemical and molecular regulation of elevated CO_2 on metabolite accumulation and utilization in Pigeonpea seeds.

Poster

Salinity driven oxidative stress in *Gerbera*

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Salinity stress is known to adversely affect a variety of plant's metabolic processes influencing its productivity and crop yield. *Gerbera jamesonii* Bolus is a commercially important ornamental plant cultivated globally in poly/net houses round the year for its cut flower production. Repeated fertigation during nethouse cultivation causes salinity leading *Gerbera* to a vulnerable stage and negatively affecting productivity/flower yield. Despite of this, little to no studies were attempted on salinity induced oxidative damage in *Gerbera*. Therefore, we verified the salt sensitivity of *Gerbera* leaf discs with varying concentrations of NaCl (0 mM–200 mM). Higher salt concentrations (above 100 mM) exhibited severe bleaching on leaf discs resulting pigmentation loss. Treatments beyond 100 mM NaCl led to a drastic decrease in total leaf chlorophyll content which might contribute to the reduction of leaf photosynthetic rate ultimately leading to the drop in productivity. Elevated lipid peroxidation and proline levels were observed upon increasing NaCl concentration. In contrast, catalase and ascorbate peroxidase activities were lowered upon treatment with higher NaCl concentrations. Overall, our results indicate that *Gerbera* is a salt sensitive species sustaining ~75 mM NaCl and beyond this could be detrimental to cellular biochemical activities. Future molecular characterization of salt-responsive genes coupled with over expression studies may shed light on developing salt tolerant *Gerbera*.

Poster

Role of *pgrl1* on high light induced change in photochemistry and super complexes in *Chlamydomonas reinhardtii*

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In photosynthetic process photosystems (PSII and PSI) are converts light energy into chemical energy through carbon fixation. Here, *Chlamydomonas reinhardtii* cells wild-type *137AH* and mutant *pgrl1* were grown under high light 250 and 500 $\mu Em^{-2}s^{-1}$ to understand the photochemical yield, super complexes organization, and protein profiling. At 250 $\mu Em^{-2}s^{-1}$ growth and the pigment accumulation was more in *137AH* compared to normal light condition (45 $\mu Em^{-2}s^{-1}$), while in 500 $\mu Em^{-2}s^{-1}$, the pigment accumulation is more in both wild-type *137AH* and *pgrl1.* Further, we have measured the chlorophyll *a* fluorescence where we observed the ETR, PSII yield has been reduced significantly in pgrl1 compare to the wild type when subjected to high light i.e. 250 and 500 $\mu Em^{-2}s^{-1}$. Additionally, we monitored the non-photochemical quenching parameters i.e., qP, qN and qL has been increased as expected that these components are involved in photoprotection. However, the F_v/F_m, variable and maximum fluorescence, quantum yield (II) were significantly decreased in mutant than the wild type in highlight. The rate of oxygen evolution reduced in high light condition demonstrates low photosynthetic activity. Furthermore, detergent solubilized thylakoids were loaded on to BN-PAGE to see the super-complexes organization, showed dissociation of mega complexes in *pgrl1,* compare to wild type 500 $\mu Em^{-2}s^{-1}$. Moreover, we noticed that PSI-LHC1, LHCs (trimer and monomer) and super complexes dissociated, the differential expression of PSII dimer and its monomer in high light indicates change in pigment-protein complexes. We also executed circular dichroism for isolated thylakoids showed reduced peak intensity which indicate the macro-aggregation of thylakoids was altered. Furthermore, protein profiling in these conditions demonstrates reduction in core proteins of PSII in *pgrl1*. These results denotes the importance of *pgrl1* and also it major role in photoprotection.

Poster

Changes of PS I supercomplexes under iron deficiency in *Chlamydomonas reingardtii*

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Iron in aerobic soils and in water mostly found as insoluble form of Fe(III) oxides, while Fe(II) is typically not found in high concentrations in the environment under aerobic conditions. On the molecular level, PSI is a prime target of iron deficiency, probably because of its high iron content (12 Fe per PSI). In the green alga *Chlamydomonas reingardtii*, iron deficiency leads not only to a pronounced degradation of PSI, but also to a remodeling of the PSI-associated LHCI, which precedes severe iron deficiency resulting in increased PSII antenna size. The present study focuses on structural and functional changes in PSI-LHCI supercomplexes under Fe deficiency in *Chlamydomonas reingardtii.* 77 K emission spectra and sucrose density gradient data show that PSI and LHCI subunits are affected under iron deficiency conditions. The visible CD data show an increase in amplitude at 675 and 656 nm, which can be attributed to a loss of tightly coupled Chl a in the LHCI antenna due to macro aggregation of the pigments. Evidence from sucrose gradients and non-denaturing (green) gels indicates that PSI-LHCI levels were reduced after cells were grown for 72 h in Fe-deficient medium. Ultrafast fluorescence spectroscopy suggests that redshifted pigments in the PSI-LHCI antenna were lost during Fe stress. Further, denaturing gel electrophoresis and immunoblot analysis reveals that levels of the PSI subunits PsaC and PsaD decreased, while PsaE was completely absent after Fe stress. The light harvesting complexes were also susceptible to iron deficiency, with Lhca1 and Lhca9 showing the most dramatic decreases [1].

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Poster

Screening for drought tolerance in lettuce using chlorophyll fluorescence imaging: limits and possibilities

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Chlorophyll fluorescence imaging (CFI) represents an important technique for screening the photosynthetic functions exposed to various environmental conditions, enabling to observe also the spatial heterogeneity of the effects. In our study, we tested opportunity to apply CFI to recognize drought sensitivity in lettuce genotypes. Eight genetically distinct parental lines of cultivated lettuce (*Lactuca sativa* L.) and one drought resistant wild lettuce (*Lactuca serriola* L.) were cultivated in a growth chamber under limited/non-limited water supply. At the end of the experiment, plants were exposed to severe drought stress by withholding of irrigation for 3 more days. CFI was recorded regularly in light exposed plants at the actinic light intensity set on the ambient level. Depending on genotypes, total dry mass in drought stressed plants decreased by 20–50% compared to control; the relative plant dry mass decrease (DMD) was used as a measure of drought sensitivity of genotypes. CFI analyses have shown a significant decrease in the apparent electron transport rate, ETR, in all genotypes (having the same trend as the efficient quantum yield, Φ_{PSII}). However, contrary to expectations, the moderate drought stress led to negligible decrease or even a slight increase of ETR, which did not correspond to the observed decrease of photosynthetic performance. It indicates that the electron transport was efficiently re-directed to alternative energy-consuming pathways, such as photorespiration and others. On the other hand, we observed a significant decrease of steady-state fluorescence intensity (F_s), both in moderate and severe drought. The steady-state fluorescence signal (F_s) reflected well the effects of water deficit on the photosynthetic apparatus better than parameter ETR, which is often referred as the most useful parameter for assessment of the photosynthetic functions. This is an important information emphasizing the need of testing the techniques in individual crops and different stress scenarios.

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Section 1.10: Systems Biology of Photosynthesis:

Integration of Genomic, Proteomic, Metabolomic and Bioinformatic Studies

Poster

Characterisation of Aureochromes – novel Blue Light photoreceptors in *Phaeodactylum tricornutum*

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Aureochromes (AUREOs) are both blue light receptors as well as transcription factor possessing a LOV and a bZIP domain. The LOV domain binds a flavin and allows light perception while the bZIP domain binds to DNA. They have only been found so far in Stramenopiles. Four orthologs of aureochromes have been identified in model diatom Phaeodactylum tricornutum, i.e. AUREO1a, 1b, 1c and AUREO2. RNAi and TALEN (Transcription activator-like effector nuclease) mediated reverse genetics approaches indicate that AUREO 1a is a repressor of high light acclimation [1, 2]. It was also shown that LOV domains can homodimerize or heterodimerize upon blue light exposure [3]. Our studies further demonstrate that the different AUREOs in P. tricornutum may have specific functions, as they do not seem to complement each other; in addition, the diel expression pattern of the aureochromes is different [3]. In this study we designed a strategy to complement AUREO1a TALEN knockout clones to verify the phenotype of the knockout mutants.

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Poster

Metabolite profiling of *Arabidopsis* seedlings subjected to UV-B

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It has been reported that the UV-B radiation (280–320 nm) stress on plants is ever-increasing as a result of stratospheric ozone depletion and it negatively influences the productivity in two-thirds of plant cultivars. Metabolomics studies on two weeks old Arabidopsis plants pointed to the reprograming of the primary and secondary metabolism[1] in response to UV-B. However, UV-B response on the metabolic profiles of early seedlings (less than 1 week old) has not been comprehensively understood which is the major focus of the current study. We subjected the wild-type Arabidopsis seedlings to four different light conditions with varying amounts of UV-B and the metabolite profiles were obtained using Gas Chromatography and Mass Spectroscopy (GC-MS). We observed several central and secondary metabolites that are both consistent and varying in response to UV-B. We will shed light on the response of UV-B on the metabolic networks in the context of our observation and existing models.

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Poster

Comparative analysis of photosynthetic gene cluster in the genus *Rhodobacter*

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Genus *Rhodobacter* belongs to anoxygenic phototrophic *Alphaproteobacteria* and are known to have versatile growth modes like photolithotrophy, photoheterotrophy and chemoheterotrophy. The Genus *Rhodobacter* encompasses 15 valid species which were isolated mostly from marine, non-marine, estuarine habitats, having different intracellular photosynthetic membranes. In the Genus *Rhodobacter* (*Rba.*) *Rba. sphaeroides* and *Rba. capsulatus* are model organisms to study the photosynthesis and carotenoid metabolism but the information related to the photosynthetic gene clusters in other species is limited. The genome sequencing of four species were carried out using Illumina HiSeq platform. In this study we are trying to correlate the isolation source, growth modes, pathways related to photosynthesis and carotenoid metabolism based on comparative genomic analysis of 13 type species of genus *Rhodobacter*. Most of the differences in the genes involved in carotenoid biosynthesis were observed whereas the bacteriochlorophyll synthesis and assembly of photosynthetic centers genes were same. This part of work will help in understanding the organization of photosynthetic gene cluster, its regulation and geospatial distribution of photosynthetic capabilities of different phototrophic bacteria.

Part 2.
Hydrogen Energy for Sustainability

Section 2.1: Energy for the Future – Hydrogen Economy

Poster

Towards Photosynthetic Hydrogen Production.

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The adverse effects of climate change can only be avoided by decarbonisation of the transport and energy sectors. Hydrogen holds a lot of promise for an alternative energy carrier, however traditional methods for large scale hydrogen production rely on fossil fuels releasing 7.33 kg CO_2/kg H_2 [1], which is both unsustainable and environmentally unfriendly. The current global market is currently estimated at $117.9 billion which is expected to grow to $152 billion by 2020 [2].

Microbes such as cyanobacteria offer a sustainable method of hydrogen production with minimal environmental impact, making them an extremely attractive proposition. Cyanobacteria can possess two functionally distinct [NiFe] hydrogenases: an uptake enzyme, only capable of hydrogen oxidation and bidirectional enzyme capable of reducing protons to evolve hydrogen [3]. *Synechocystis* sp. PCC 6803 encodes one bidirectional [NiFe] which is a heteropentameric enzyme composed of a hydrogenase module (HoxH & HoxY), forming the catalytic core and the diaphorase module (HoxE, HoxF & HoxU).

We have previously demonstrated that the hydrogenase of *Synechocystis* sp. PCC 6803 is thylakoid associated. There are two distinct hydrogenase populations, one dispersed throughout the thylakoid and the other forming distinct puncta which correlate with hydrogen evolution [4]. We are currently investigating co-localisation of the hydrogenase complex with potential partners from the electron transport chain.

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Section 2.3: Biological hydrogen production

Lecture

Biohydrogen production in the nexus of acidogenesis and photosynthesis: Lab to pilot scale studies

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Realizing the necessity, the world is gradually shifting from fossil-based linear economy to bio-based circular economy. Valorizing of waste is emerging interest in the modern bio-economies. In this context, hydrogen is gaining significant attention as a future energy carrier. Considerable interest has been observed on biohydrogen production through biological routes viz., Fermentation, Photobiological, Enzymatic, Thermochemical, etc. contrary to fossil-based routes. Using waste as a resource/feedstock for biohydrogen generation has instigated considerable interest and further opening up a new avenue for the utilization of these inexhaustible and renewable energy sources. Recently, we commissioned a state of art pilot plant facility (10,000 liters operation capacity) for the production of biohydrogen (50,000 liters/day) from waste/wastewater. The pilot plant has acidogenic reactor inter-connected with seven unit operations each with a defined function i.e. inoculum preparation, redox control, buffering/pre-treatment, biogas holding, anaerobic digestion, auto biogas-flare and water/waste feeding. This communication elaborates the lab to pilot scale studies with the aim of scaling up the technology towards commercialization. Environmental sustainability and economic viability can be envisaged by considering the integrated approach. Biorefinery platform structuring acidogenic process at the focal point and sequentially integrating multiple bioprocesses including photosynthesis towards the production of various biobased products in a unified approach will also be discussed in the context of circular bioeconomy.

Poster

Elucidating the role of native electron transport chain in extracellular electron transport (EET) towards bioelectricity and biohydrogen production

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Microbial electrochemical systems (MES) can function as renewable and sustainable option for converting organic waste to bio-energy. In MES, transport of electrons from bacteria to an electrode is the key to its functioning, but the mechanism by which bacteria extracellularly transport electrons is unknown. A single chambered MES using FTO plate as anode and SS316 mesh as cathode was used to study the role of NADH dehydrogenase II (*ndh2*) in extracellular transport (EET) further leading to enhancement of biohydrogen and bioelectricity. To understand this, *ndh2* gene was over-expressed in *E. coli* while chronoamperometry, cyclic voltammetry and iron reduction assays were used to confirm the EET. Protein was affinity purified and western blotting was used to confirm the localization into membrane. Bio-physical techniques were used to understand the structure of the protein and it was further studied by using protein film voltammetry. The result of the study has confirmed that the NADH DH2 has role in EET which can be used to enhance the production of bioelectricity and biohydrogen in MES.

Poster

In vitro metabolic engineering strategies towards H_2 production – feasibility assessment in Indian context

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In vitro metabolic engineering methods using synthetic enzyme pathways is successfully demonstrated as a strategy for high hydrogen (H_2) yields. Mainly, it has been shown that it is feasible to convert the renewable sources of biomass waste (Cellulose and starch) and water to hydrogen gas using a series of enzymes in the laboratory conditions [1, 2]. While the strategy appears promising, it needs thorough feasibility assessment in the Indian context which is the main focus of this work. We will briefly review the current in-vivo strategies adapted by various groups towards H_2 production. In addition, we will present the cost assessment, potential alternate *in vitro* strategies and the feasibility of this technology in the context of renewable raw material available in India. This introductory study will provide the platform to adapt and improvise further studies in the context of H_2 economy.

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Poster

Effects of nutrients, titanium dioxide nanoparticle and sodium bisulphite on biological hydrogen production from *Chlorococcum minutum*

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Renewable energy is one of the primary concerns in any country. Moreover production of renewable energy such as biological hydrogen or biohydrogen (H_2) from primitive plants such as algae is gathered momentum recently. In this process, our laboratory focused on an interesting fresh water green alga *Chlorococcum minutum* belongs to chlorophyceae for hydrogen production. We performed our experiments with this alga using different strengths of TAP nutrients and other factors such as nanoparticle titanium dioxide (nano-TiO_2) and Sodium bisulphite ($NaHSO_3$) under *in vitro* conditions. Full strength of TAP medium proved as beneficial for improvement of cell growth as well H_2 production when compare to quarter and half strength media. It is well-known fact that full strength nutrients are essential for growth and development and proved in many occasions. In addition we studied the impact of nano-TiO_2 and $NaHSO_3$ independently along with TAP medium on H_2 production in this species. Generally nano-TiO_2 semiconductor exhibits photocatalytic activity for H_2 generation from water molecules. We used different concentrations of nano-TiO_2 and found that 20 µg is optimum for efficient H_2 generation. Similarly a low concentration (0.4 mM) of $NaHSO_3$ is also useful for the enhancement of H_2 production, whereas high concentrations often inhibit photosynthesis and hydrogen production. $NaHSO_3$ functions as oxygen scavenger in algal cultures and create anoxic condition which is an essential step for hydrogenase function. From this preliminary study we concluded that full strength TAP, 20 µg of nano-TiO_2 and 0.4 mM of $NaHSO_3$ were useful for improvement of photobiological H_2 production in this alga and future works may answer some more clarifications.

Poster

Improvement of gas-liquid mass transfer increases hydrogen production by microbial water-gas shift reaction with economical process

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Hydrogen can be considered as a clean energy with high energy contents (122 kJ/g) and low polluting fuel that can be used for transportation, heating and power generation [1]. One of the hydrogen production ways, biological hydrogen production is a cheap method for its mild operation condition. Furthermore, we used stirred-less reactor for reduction of aeration cost and LDG (Lindz-Donawitz Converter Gas) gas which is by-product gas in ironworks for water-gas shift reaction. Hyperthermophilic archaeon, *Thermococcus onnurineus* NA1, was reported that it was isolated from deep sea (depth, 1650 m), hence, it was high pressure-resistant microorganism [2]. In gas fermentation, gas-liquid mass transfer is main bottle-neck and applying high pressure and microbubble distribution will increase mass transfer. Hence, pressurized bioreactor and small pore size of sparger were conducted to high hydrogen productivity. The *T. onnurineus* NA1 (KCTC10859) strain was cultivated in a 20 L bioreactor equipped with 3 um and 1–1.5 mm pore size of sparger and atmosphere to 7 bar of pressure condition at 80°C. For cell growth and hydrogen production, a simulated LDG with 60% of CO and 40% of N_2 was fed to bioreactor. In this study, developed mass transfer bioreactor system was conducted via microbial water gas shift process. Increased CO mass transfer can affect with increasing hydrogen production by high pressure-resistant microorganism, *T. onnurineus NA1*. The specific highest hydrogen productivity was conducted 447 mmol/L/h in this system.

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Section 2.4: Hydrogenases

Lecture

[NiFe]-Hydrogenase maturation and subunit assembly

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[NiFe]-hydrogenases are ancient metalloenzymes that catalyse the reversible oxidation of dihydrogen. All [NiFe]-hydrogenases are composed of minimally a catalytic large subunit with a $NiFe(CN)_2CO$-cofactor in the active site and an electron-transferring small subunit, which has an array of iron-sulphur clusters that relay electrons to and from the large subunit; often the enzymes are anchored in the cytoplasmic membrane. The $NiFe(CN)_2CO$ cofactor in the active site of the large subunit is synthesized by a highly conserved set of six Hyp (hydrogenase pleiotropy- HypA through F) proteins that are common to all organisms that have [NiFe]-hydrogenases. The $Fe(CN)_2CO$ moiety of the active site cofactor is assembled on a Hyp-protein scaffold. The diatomic ligands CO and CN^- are derived from distinct intracellular metabolic precursors, with both cyanyl moieties being derived from carbamoylphosphate. The precise mechanism of CO ligand synthesis is still unclear, however, current evidence supports a hypothesis whereby the precursor is CO_2 bound to an iron ion. After attachment of the cyanyl groups to Fe(I)-CO, the completed moiety is inserted into the apo-form of the large subunit. Introduction of the nickel ion is catalysed by the HypAB complex, assisted by the peptidyl-prolyl *cis/trans* isomerase SlyD, and this occurs after $Fe(CN)_2CO$ group insertion. Active site synthesis is completed by an endoprotease cleavage event that removes a *C*-terminal peptide from the large subunit, resulting in a conformational switch that closes the active site. Only after the conformational change has occurred does the large subunit interact with the mature small subunit, which has received its full complement of iron-sulphur clusters through the action of the Isc (iron-sulphur cluster) biosynthetic machinery. The heterodimer is then delivered to its final cellular destination, which can be the cytoplasm, as in the case of soluble hydrogenases, or the cytoplasmic membrane, in the case of H_2-oxidizing or H_2-producing enzymes. If it is the latter destination, then translocation of the large-small subunit heterodimer is performed by the Tat (twin-arginine translocation) machinery, which recognizes a Tat-signal peptide, which is located on the small subunit. After membrane translocation the heterodimer interacts with the membrane-anchor subunit, which is inserted into the membrane separately. How this highly orchestrated series of events is coordinated to achieve correct assembly of an active [NiFe]-hydrogenase will be discussed.

Lecture

Hydrogenase electrode based on HydSL hydrogenase from *Thiocasa roseopersicina* with high current density

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Biofuel cells are a type of fuel cell that utilizes not noble metals but enzymes as the electrocatalysts to catalyze the oxidation of hydrogen and the reduction of oxygen for energy conversion to electricity. Due to high cost of noble metals it is desirable replacing them by enzymes.

Hydrogenases are metalloenzymes that catalyze the activation of molecular hydrogen. There are three types of hydrogenases differing in the content of metals in their active site: [NiFe]-hydrogenases; [FeFe]-hydrogenases; Fe-hydrogenases with a Fe-containing cofactor. For most [NiFe]-hydrogenases, the bimetallic center was shown to be covalently bound to the protein by four cysteine residues, two of which form a connecting bridge between Ni and Fe, and the other two coordinate Ni. The Fe ion is associated with diatomic ligands, one CO ligand and two CN^- ligands.

NiFe-hydrogenase HydSL from purple sulfur bacterium *Thiocapsa roseopersicina* BBS belongs to group 1, hydrogen-uptake hydrogenases. It was shown that this enzyme is capable of electrocatalytic hydrogen uptake when immobilized on electrode surface. However, hydrogen electrodes based on this hydrogenase produce low current density comparing to Pt electrodes (not more than 1.5 mA cm^{-2}). This is the main problem preventing commercial application of this enzyme in fuel cells.

We measured activation energy in reaction of electrocatalytic hydrogen uptake by hydrogen electrode based on HydSL hydrogenase from *T. roseopersicina* as a function of overvoltage. With an increase of overvoltage from 5 to 150 mV, the activation energy decreased from 19 to 6 kJ mol^{-1}. The activation energy of electrocatalysis was equal to 13 kJ mol^{-1} (the activation energy of HydSL hydrogenase) only at overvoltage 35 mV. Around this overvoltage we did not see any stabilization of activation energy of electrocatalysis. One could conclude that at different overvoltage, we have different limiting steps of electrocatalysis and that hydrogenase is not limiting at any overvoltage.

We constructed particular cell for electrochemical H_2 uptake where hydrogen electrode has direct connection with H_2 gas without solution. Several samples of hydrogenase electrode gave 3–15 mA cm^{-2} current density at 15 mV of overvoltage, which is several degrees higher than previous published data with HydSL hydrogenase.

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Section 2.5: Proton Reduction Catalysts

Poster

Photovoltaic performance and influence of organo metal halide electrolyte on chlorophyll-protein complex nano particles based solar cells

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According to the molecular reaction mechanism of photosynthesis in plants, excited chlorophyll protein releases electrons in the presence of sunlight. The excited chlorophyll electrons are transferred to electron deficient molecule; hence a potential difference is generated and electricity will be generated which can be detected when allowed to pass through a circuit. Previous reports on solar cell are built using chlorophyll protein Nano particles (n-type), fullerene nanoparticles (p-type), KI/I_2 as electrolyte solution, to transfer electrons on silver coated glass slides. In the present study, the synthesis of methyl ammonium lead iodide electrolyte is compared with KI/I_2 in solar conversion efficiency progress. The transfer of electrons and thereby production of electricity is done using nanoparticles of chlorophyll protein complexes isolated from different plant leaves. We noticed that, in the presence of sunlight, photons strike chlorophyll, and then excited electrons transport through the methyl ammonium lead iodide perovskite electrolyte transport layer which is much faster than the KI/I_2 electrolyte in the chlorophyll-protein complex Nano particles based solar cells. In addition, it has been noticed that in the presence of methyl ammonium lead iodide, an increase in voltage in the range of few millivolts, solar power conversion under photovoltaic effect results in the release of excited electrons, moving from photo excited chlorophyll (n-type) to fullerene nanoparticle (p-type) electron sink, and current flows from fullerene(p-type) to chlorophyll (n-type).

Section 2.7: Artificial Photosynthesis for Hydrogen energy

Lecture

Temperature-sensitive PSII: A novel approach for sustainable photosynthetic hydrogen production

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The development of technology for sustainable hydrogen production is crucial for a global transition to a clean and sustainable energy economy. Certain microalgae and cyanobacteria express a [FeFe]-hydrogenase which evolves H_2 by using electrons derived from photosynthetic water splitting. Photolysis of H_2O into H_2 and O_2 represents the most sustainable hydrogen production method. However, O_2 inhibits the [FeFe]-hydrogenase and highly complicates obtaining pure H_2. We suggested that a cyclic temperature change regime could allow for temporal separation of H_2 and O_2 production. This system requires a temperature sensitive photosystem II mutant, which did not exist until now. To this aim, we generated such mutants in a well-studied model organism for green microalgae (*Chlamydomonas reinhardtii*) and identified the mutations in order to be engineered into a suitable target organism for large-scale H_2 production. We randomly mutagenized *C. reinhardtii* cells and screened for mutants which exhibited temperature-sensitive photoautotrophic growth. The selected mutants were characterized by their ability to evolve oxygen and hydrogen at 25 and 37°C. This enabled identifying mutants with the ability of temporally inactivating O_2 production at high temperature for continuous H_2 production while resuming O_2 production at low temperature. Upon further characterization by spectroscopy and biomolecular means, four mutants revealed to be adequate candidates for the proposed cyclic hydrogen production system. These mutants were genotyped by crossing and whole genome sequencing, facilitating the transfer of this technology to alternative target organisms. These mutant microalgae represent a feasible approach for a large-scale hydrogen production plant.

Poster

Photochemical hydrogen evolution with cyanobacterial photosystem I–platinum nanoparticle hybrid systems

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One of the ways to achieve artificial photosynthesis in a high quantum yield is to combine photosynthetic proteins and inorganic catalysts. Photosystem I (PS I) of plants and algae is a promising material for solar hydrogen generation, since it produces highly reducing photo-excited electrons under the irradiation of visible light. In this study, cyanobacterial photosystem I was conjugated with hydrogen evolving platinum nanoparticles by replacing vitamin K_1 (VK_1) molecules inside PS I with VK_1-mimicking molecular wires which had a platinum nanoparticle (PtNP) at their end. This process is called "reconstitution" [1], and was confirmed by the formation of oxidized P700 chlorophyll special pairs during the light irradiation. PS I–PtNP complex produced hydrogen at a rate of 0.026 mol H_2 (mol PS I)$^{-1}$ h^{-1} after illumination for 24 h, when sodium ascorbate was used as a sacrificial reagent and DCIP as an electron mediator. One of the biomolecular electron mediators, cytochrome *c*, enhanced the hydrogen evolution rate up to 0.053 mol H_2 (mol PS I)$^{-1}$ h^{-1}.[2]

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Poster

Cocatalyst free Z-schematic enhanced H_2 evolution over $LaVO_4/BiVO_4$ composite photocatalyst using Ag as an electron mediator

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A novel cocatalyst free Z-schematic photocatalytic system of $Ag/LaVO_4/BiVO_4$ was successfully fabricated for clean hydrogen fuel evolution inspired by the Z-scheme water splitting mimicking photosynthesis of green plants. The spherical nanoparticles of $LaVO_4$ were prepared in solution combustion method for the first time using glycine as a fuel, $BiVO_4$ was deposited onto $LaVO_4$ through a deposition–precipitation method and Ag was loaded on the surface of $LaVO_4/BiVO_4$ composite by photoreduction method. The composites were characterized by XRD, UV-vis DRS, SEM, TEM, EDS and XPS to ensure the successful integration of Ag or (and) $BiVO_4$ with $LaVO_4$. A series of photocatalytic H_2 evolution experiments, employing Na_2S and Na_2SO_3 as hole scavengers, showed that the $Ag/LaVO_4/BiVO_4$ composite exhibited a superior photocatalytic performance compared to single $LaVO_4$ or $BiVO_4$. Although $BiVO_4$ cannot be used for H_2 evolution, it can significantly enhance the H_2 evolution performance of $LaVO_4$ through a Z-scheme mechanism with Ag as an electron mediator. Moreover, investigations on photoluminescence and fluorescence lifetime measurements demonstrated the greater separation efficacy of photoinduced excitons in the Z-scheme $Ag/LaVO_4/BiVO_4$ photocatalytic system. This newly constructed $LaVO_4$ based Z-scheme system exhibits promising photocatalytic H_2 evolution activity with significant longevity and will be useful for potential applications in energy driven technologies.

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