Basic Information

- **Project No. and Title:** NC1200: Regulation of Photosynthetic Processes
- **Period Covered:** 11/01/2018 to 11/30/2018
- **Date of Report:**
- **Annual Meeting Dates:** 11/22/2019 to 11/24/2019

Participants

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Brief Summary of Minutes of Annual Meeting

The meeting was held at Washington State University Downtown Seattle Conference Center, 901 Fifth Ave, Seattle, WA. Asaph Cousins (WSU) began the meeting with introductions. The three guests were introduced (Zhanguo Xin – USDA-ARS; Nicole Buan – UNL and Henning Kunz – WSU).

Jeff Harper, University of Nevada (Title: *Calcium signals, salicylic acid, and plant growth*) spoke about calcium movement in plants addressing that blue light causes calcium spiking. At least three calcium circuits exist in plant cells including the PM, ER and the vacuole. A set of P-type ATPase pump calcium across the PM, ER membrane and tonoplast. Knock-out of a cluster of PM P-type ATPases results in plants with severe growth phenotype, increased vulnerability to diseases and sterility. Knock-out of vacuolar P-type ATPases leads also to plants with growth phenotype and leaf lesions, whereas the knock-out of ER ATPases leads to leaf lesions only. It is hypothesized that lesisons are caused by altered salicylic acid (SA) levels triggering cell death. The Flg22 pathogen elicitor peptide triggers Ca waves in WT and aca1/2/7 3X KO. The Ca transients are bigger and longer in aca 1/2/6 3X KO than in wt. NahG degrades SA, suppresses lesion formation and lead to bigger plants. However, it is assumed that these larger plants are more susceptible to stresses. The information content of Ca signals can be shaped by Ca pumps. It is hypothesized that less Ca pump activity leads to more SA and poor poor plant growth. In turn increasing Ca pump activity would lead to larger plants.

Anastasios Melis, University of California, Berkeley (Title: *Fusion constructs as protein overexpression vectors*) spoke about biotechnological production of isoprenoids like isoprene, phellandrene (monoterpene in cosmetics industry), and geranyl linalool (diterpenol, fragrance, feedstock for an antimalarial drug) in cyanobacteria. The goal is to produce as much isoprenoids as possible (measured as isoprenoid vs biomass). The strategy is to transfer plant genes into cyanobacteria (codon optimized) but the isoprenoid yield was very low (100 - 150 ug/ g biomass). Two problems were identified: 1. Slow rates of isoprenoid enzymatic catalysis (kcat Isoprene synthase of 4.4 s-1), 2. Protein expression is very low (not visible in SDS-PAGE). Solution – Overexpress of the enzymes at the same levels as Rubsico by using strong promotors. But this did not improve expression. Then the operon structure was targeted. CpcB and CpcA were removed and the Cpc promoter overexpressed in the gene cassette (without terpene synthases). It was concluded that a problem with the terpenoid synthase systems exists. Fusion of cpcB with the terpenoid synthase leads to much higher (10x) protein expression (most abundant protein). Further optimizations lead to further increase in isoprenoid yield (24 mg / g dry cell weight).

Ru Zhang, Donald Danforth Plant Science Center (Title: *Understanding Heat Tolerance in Photosynthetic Cells by Using the Green Alga* Chlamydomonas reinhardtii) spoke about using Chlamydomonas as a model to study heat responses in photosynthetic organisms. Outdoor algal ponds experience high temperatures up to 35C. Chlamy can be primed to 35C resulting in acquired thermotolerance and heat stress memory. A systems levels approach was applied to investigated how Chlamy responds to 35C or 40C. Samples were taken at different timepoints. The growth rates are reduced during high temperature periods. Cells become bigger at 35C and much bigger at 40C that can be

explained by inhibition of cell cycle progression. Furthermore at 40C but not 35C O2 evolution rates and respiration rates was reduced. 40C treatment induced higher NPQ than 35C treatment. Initial high rates are decreased by the end of 24h treatment. The NPQ is more sensitive to proton motive force at both 35C and 40C (monitored by electrochromic shift). A regulatory network will be established by computational analysis of omics data (genomics, transcriptomics, proteomics, metabolomics) that will lead to a prioritizes heat-gene-list. It was found that the responses of Chlamy to heat stresses are dynamic, i.e. cells respond differently to moderate and acute heat stress.

Thomas Sharkey, Michigan State University (Title: *The source of carbon in RL (aka Rd)*) spoke about how to estimate and understand the oxygen consumption (respiratory) parameter in the light (RL). Net CO2 assimilation A is defined as: A = vc (velocity of carboxylation by rubisco) -0.5*Vo (velocity of oxygenation by rubisco) -Rd (the rate of mitochondrial respiration in the light). RL can be measured by the Laisk method plotting A vs.vc or the Kok method (A vs light response curve). Yin suggest using photosynthetic efficiency. Loreto method to measure RL is by evolution of $^{12}CO_2$ into 99% $^{13}CO_2$ atmosphere. RL gets determined by combined gas exchange and chlorophyll fluorescence measurements (CO2 assimilation and linear electron transport). However, there is a blue light effect for measuring LEF caused by carotenoids (act as energy quencher). RL measured isotopically accounts for about 1/4 of the RL that can be measured by gas exchange and fluorescence. Other sinks for assimilated CO_2 should be considered: PDH/TCA cycle, FA synthesis, Isoprenoid synthesis, TCA cycle and glycolysis are inhibited in the light 20-fold. In the Calvin-Benson cycle, the glucose-6-phosphate (cytoplasmic) shunt could be used. This would burn ATP, thus affecting the electron transport rate, supported by G6PDH loss of function mutants that have reduced RL. The G6PDH shunt could explain slow C13 labeling results: Glucose-6-phosphate is in cytosol (slow label) and stroma (fast label). The slow-to-label pool of carbon contributes to RL. The unlabeled carbon from the cytosol get re-injected into Calvin-Benson via Ru5P. Calculations suggest the shunt is accounting for about 80% of RL (50% is labeled carbon, 33% is unlabeled carbon. 20% of other things e.g. fatty acid synthesis). Ramifications: A "high cyclic" mutant hcef1 was found to lack stromal FBPase. That means that F6P can turn into G6P in the chloroplast or the cytoplasm. Respiration in the light is high in photorespiration mutants. Increased RL is accompanied by increased cyclic electron flow.

Rob Aiken, Kansas State University (Title: *Three perspectives on photosynthetic light utilization*) spoke about the possibility whether gain in biomass productivity can be transferred into increased yield. It was indeed found that increase in above-ground biomass translates well to grain yield. Mostly this shows that for well-watered plants grain yield is equivalent to dry grain yield. A strategy to increase yield is truncation of the light harvesting apparatus (TLA) allowing better light penetration into plant canopies by higher light transmission through the leaf to lower layers. It was shown that this leads to biomass increases by about 25%. The objective of the study is to identify grain sorghum lines with the TLA trait in the TX623 mutant library. The approach was visual inspection, measure chlorophyll a : b ratio, determination of antenna size, back crosses. No light green phenotypes in 2019 mutation observation lines. Found high chlorophyll a : b ratios (11:1 to 27:1). Gas exchange was normal, fluorescence data was weird. The explanation for the missing phenotype for TLA mutants is that a special herbicide was sprayed (huskie post-emergent herbicide), that inhibits HPPD and carotenoid biosynthesis.

Christoph Benning, Michigan State University (Title: *Thylakoid lipid metabolism in a changing environment*) spoke about the plant lipidome to determining the function of lipids in photosynthesis. A particular question is how plastidial phosphatidic acid (PA) and diacylglycerol (DAG) get to the MGD1 enzyme that catalyzes the formation of the chloroplast lipid monogalactosyldiacylglycerol (MGDG). One of the clues comes from rbl10 mutants. These are affected in PA metabolism. It is an integral membrane protein that appears to be a rhomboid protease. The mutants accumulate PA. Pulse chase experiments show high labeling of PA and low labeling of MGDG that implies that PA trafficking to MGDG is less effective. The RBL10 seems to be autocatalytic towards its own C-terminus and is part of a large protein (660 kDa) complex. Co-IP and split ubiquitin Y2H identifies more than 20 candidate interacting proteins. It is hypothesized that assembly/stability of larger complex is RBL10. In another project the role of redox active peroxiredoxin (PRXQ) for FAD4 activity involved in forming the specific lipid 16:1delta3t PG is examined. In the cold (6 C), prxq and fad4 mutants have altered photosynthetic activity and ATPase and SBPase are more reduced. It is hypothesized that 16:1delta3 PG could be involved in ROS signaling by scavenging ROS from the light harvesting antenna.

Helmut Kirchhoff, Washington State University (Title: *More than 60 Years of Photosynthesis Research: How do PSII and PSI Functionally Connect in Higher Plants?*) spoke about whether the mobile electron carriers plastoquinone (PQ) or plastocyann (PC) connect both photosystems in higher plants that are separated by several 100 nm to stacked and unstacked thylakoid domains. The challenge for long-range diffusion PQ and PC is that diffusion spaces for them are crowded by protein complexes that might require architectural dynamics. To address the question of long-range electron transport in photosynthesis the CURT1abcd knock-out mutant was employed that have drastically increased diameter of stacked areas (from 400 nm in wt to 1600 nm). The research idea is that extending the diffusion distance between PSII and PSI allows detection of the long-range carrier. In vivo spectroscopy probing

either PQ or PC diffusion reveals that PC diffusion is impaired in the curt1 mutant giving evidence that this is the long-range electron carrier. Dynamic swelling and shrinkage of the thylakoid lumen could control the mobility of PC and therefore linear electron transport.

Rebecca Roston, NE-AES (Title: *Plant growth and non-photochemical quenching improve in the presence of an archaeal antioxidant*) spoke about an antioxidant supplementation was tested under multiple growth conditions. Media and soil growth of *A. thaliana* supplemented with archaeal antioxidants or sprayed with archaeal antioxidants grows better than controls. The same is true for *O. basilicum* grown hydroponically and on soil. The addition of antioxidants does not seem to disrupt “normal” redox signaling, because germination is normal, as is root patterning. It seems that the archaeal antioxidants are metabolized because they can be used to supply *A. thaliana* with S in media that lacks it. Further evidence that the archaeal antioxidant is effective in areal tissues is when applied through the media, it reduces the cytoplasm of leaf epidermal cells. Finally, the antioxidant seems to improve the ability of the plant to disperse excess light energy through non-photochemical quenching.

Henning Kunz, Washington State University (Title: *The chloroplast ion homeostasis is critical for plastome gene expression*) spoke about the interaction between potassium concentration in chloroplasts and plastid gene expression. About 95% of plastid proteins are encoded in the nucleus whereas 5% remained in the plastid. K+ transport across chloroplast envelope membrane is mediated by KEA1 and KEA2. Trypsin treatments of isolated plastids reveal the basic topology of KEA1/2. Antibodies against the N-terminus of the two K+ transporters were generated tagged at the C-term. Expression of KEA1 and KEA2 in the double KO background reveals N-term and C-term both face the stroma. KO plants of kea1/kea2 are pale with a photosynthetic phenotype, but recover when they are older. Younger leaves over accumulate potassium. The mutant takes up much more rubidium. If grown under high salt the photosynthetic phenotype partially recoved. Nuclear transcriptomics from kea1 and kea2 mutants show an enrichment in genes related to plastid rRNA processing. These are dramatically different in mutant and wildtype. RNA binding proteins from the nucleus are required to process chloroplast genes into proteins. In double KO kea1kea2, TIC/TOC increases, photosynthetic proteins decrease. Kea1kea2 fails to express plastome encoded proteins in the young leaves.

Katarzyna Glowacka, NE-AES (Title: *Natural variation in kinetics of non-photochemical quenching of C_4 grasses*) spoke about how the non-photochemical quenching mechanism depend on developmental stages in corn plants and on N-treatments. It was found that NPQ as well as Fv and Fm varies both under N-treatments and with the plant development. The NPQ values for soft stem versus non-soft stem tissues are different. Crosses of these populations yields F1 heterosis. The soft stem groups are very different in NPQ, they have a faster relaxation of NPQ. The results indicate that chlorophyll fluorescence can be used to determine which lines of corn can be crossed to generate heterosis.

Fred Below, IL-AES (Title: *Increasing light interception, canopy photosynthesis, and yield of corn with narrow rows*) spoke about research of how row spacing of corn plants determine crop yield. Researching 20" rows vs 30" rows. Planting 44,000 plants per acre in 30 inch rows makes a plant every 4.8" whereas at 20 inch rows plants are separated by >7 inches. How does the spatial arrangements change the phenotypic traits that hybrids possess? In 2017-2018 different hybrids spaced at 20" vs 30" very tested for various locations. Results: (i) Height: hybrid V8 shows no difference in height whereas hybrid R1 at 20" spacing all are taller, with higher density, they get shorter. (ii) Leaf area: In 20" rows, the leaf area is larger per plant at every density planted. (iii) Leaf area index (leaf area per ground area): Still higher for the 20" row. (iv) Hybrid variation in leaf angle (vary from 66 - 70 degrees): Roots get smaller when plants get smaller. (v) Roots of 20" plant are always bigger. Some hybrids like the narrow rows better than others. 43 plant traits were measured to identify what determines tolerance to density in rows. 14 trait model were identified that described the tolerance to row traits. Most responsive traits are: plant height, stem diameter, biomass, thinner leaves and more upright leaf angle. Low specific leaf weight, high leaf area index. Narrow rows are a way to increase light interception and manage a higher population of corn plants.

Zhanguo Xin, USDA-ARS (Title: *Sorghum pedigreed mutant library as a resource for dissecting the regulatory processes of photosynthesis*) spoke about a pedigreed mutant library in Sorghum. The library contains >10,000 individual M2 seed pools >6,400 M3 seeds obtained. High quality DNA was prepared for all lines. 256 lines were sequenced. The phenotypes are dramatically different: For examples dwarf plants>200 lines, male sterile plants >200 lines, plants with more erected leaf 83 lines. Over 35,270 SNPs genes were recorded. A free public domain software 'Cyverse' was presented that gives a picture of genomic positions of mutations and suggest mutations for a certain trait.

Asaph Cousins, Washington State University (Title: *Leaf anatomy and mesophyll CO_2 conductance in C_4 grasses*) spoke about C_4 plants with different leaf conductance. In C_3 plants photosynthesis CO_2 goes from stomata through cell wall, the plasma membrane and chloroplast envelope into chloroplasts. Concomitantly, there is H_2O loss. C_4

compartmentalizes CO_2 fixation so that H_2O loss is minimized that is realized by a special C_4 leaf anatomy that supplies CO_2 to bundle sheath. Significant leaf anatomical differences exist in bundle sheath cells. How does this anatomical variation influence CO_2 retention? To study this the leaf CO_2 conductance is plotted against net rate of CO_2 efficiency. The ratio of stomal density on the bottom of the leaf has a stronger correlation with conductance than those on the top. The best correlation is with the paths that the CO_2 takes inside the leaf. This is primarily driven by mesophyll cell surface area. Future research will examine leaf hydraulic conductance and CO_2 conductance by using three-dimensional leaf structure.

Business meeting minutes:

Location of next meeting 2020: Will be in Michigan hosted by Christoph Benning and Tom Sharkey.
Timing? This time of year would be okay, so the just-before thanksgiving timeline looks good. They will communicate available times with us soon. Venue: Henry center with hotel attached and University club attached. Fly to Lansing or Detroit. Recommend flying to Detroit and book the bus from the airport.

Location of meeting in 2021: Will also be the lead for the renewal grant.
The grant is due in the fall of 2021, it will be decided in the spring of 2022.
It would be good to separate the two jobs (hosting and writing) too.
Christoph Benning is not willing to be the author, as he is academic advisor.
Rebecca Roston volunteers for hosting of the 2021 meeting in Nebraska.
Christoph Benning suggests we form a writing committee next year- tasks will be assigned. We will decide the objectives and assign a person for each objective and to oversee. Details will be discussed at next meeting. We could ask people to commit for two out of the five meetings for the renewal. This is a committed group.
Confirm Zhanguo Xin is accepted unanimous as a new member. Movement made by Tom Sharkey, Fred Below seconded, no dissenting votes.

Christoph Benning gave a presentation that discusses this meeting. We discussed some of the following topics from the presentation:

Multistate projects-
North Central region (in 2019) ~ 45 NCs, 22 ERAs, 15 Coordinating Committees, 3 DCs 10 Acs, 0Rapid Responses, 7 National Research Reports

Reporting
Annual Midterm and Final (we are at Midterm): Includes impacts and next steps, rather than a sum of individual activities. Collaboration and synergy are emphasized.
NRSPs (off-the-top (OTT) funding) - that means that when we participate in this, it keeps the money coming into our AESs. This is the same for several groups.

Participant Benefits-

Committee Responsibilities-

Report Content highlights-
Minutes as notes tied to the Agenda, not detailed, attendees, report focuses on 1-3 impacts, Synergistics and project objectives, publications and grants awarded.

Project Reviews-
5 year project deadlines and timelines
9/15 Request to write a proposal with AA identified
10/15 upload objectives
11/15 Ideally all participants added by AES office in App E
12/1 AA review due

Accomplishments

Activities:

Objective 1. Identify Strategies to optimize the assembly and function of the photosynthetic membrane.

- We continued the analysis of the reaction mechanism, activation, and function of the unusual FAD4 desaturase of Arabidopsis. FAD4 is responsible for the formation of a phosphatidylglycerol species in chloroplasts that contains a 16:1 delta 3 trans fatty acid at its sn-2 position. We identified a new protein cofactor, a specific peroxiredoxin, required for the activity of this protein in vivo and in vitro. We were able to reconstitute the desaturase and its cofactor in yeast microsomes. Specific cysteine residues in FAD4 and peroxiredoxin were identified that are essential for activation and activity of FAD4 activity. Based on these results, we hypothesize that the redox state

of the chloroplast is linked with the activity of the FAD4 desaturase as a photoprotective mechanism under certain abiotic stress conditions. A manuscript describing these findings is under review.

- We advanced the analysis of a novel rhomboid protease of Arabidopsis located in the inner chloroplast envelope membrane. We published the biochemical analysis of the respective mutant, in which the supply of lipid precursors, i.e. phosphatidic acid, from the inside of the chloroplast to the enzyme that assembles the galactolipid monogalactosyldiacylglycerol is disrupted, while the precursor supply from the ER compensates. We have made progress in determining that the respective rhomboid protease has catalytic activity, as we can demonstrate that it autocatalytically cleaves off its C-terminus, but we do not know at this time the relevance of this cleavage. The protein is associated with a large complex of the inner chloroplast envelope membrane and we are investigating its composition. We are raising antibodies against this protein to aide in this process.
- The analysis of the rhomboid protease described above raised new questions about phosphatidic acid movement and metabolism in the inner envelope membrane. For example, which proteins participate in the conversion of phosphatidic acid to diacylglycerol, the precursor of galactolipid biosynthesis, remains unclear. We are currently studying three candidates encoding phosphatidic acid phosphatases and their respective loss-of-function mutants alone and in combinations and we identified a double mutant that shows strong growth reduction, which is further studied. The proteins have been previously shown to be in the chloroplast, but their exact membrane association and topology is critical for our understanding of phosphatidic acid metabolism and is currently under investigation.
- We have established quantitative tools to determine the abundance of photosynthetic protein complexes in stacked and unstacked thylakoid domains. This paves the way to study dynamics in lateral protein reorganizations under different light conditions and serve as input information for coarse grain computer simulations. This thylakoid fractionation platform was employed to study the light-induced redistribution of cytochrome b6f complexes in wildtype and mutants affected in regulation of photosynthetic energy conversion (kinase, phosphatase, and cyclic electron transport). Finally, thylakoid ion channel and transporter mutants that are likely involved in thylakoid swelling/shrinkage dynamics were characterized leading to a mathematic model of ion flux and thylakoid membrane energization.

Objective 2. Identify strategies to modify biochemical and regulatory factors that impact the photosynthetic capture and photorespiratory release of CO_2.

- We performed omics using the model green alga *Chlamydomonas reinhardtii* to investigate the system-wide changes of transcripts, proteins and pathways in response to moderate and acute heat stress;
- We performed genome-wide screens using the *Chlamydomonas* mutant library to identify genes with potential roles in heat responses;
- We studied the non-photochemical quenching (NPQ) pathway and Rubisco activase in C_4 plants by using *Setaria Viridis* as a model;
- We investigated the possible mechanisms of cold and salt tolerance of the Antarctic green alga *Chlamydomonas* sp. UWO241 (UWO241) which has sustained cyclic electron flow (CEF) around photosystem I (PSI).
- For C_4 photosynthesis, a specific phospho*enol*pyruvate carboxylase (PEPC) isozyme evolved from a non-photosynthetic PEPC to power the C_4 carbon concentrating mechanism. It has been hypothesized that an increase in the affinity of PEPC for HCO_3^- (K_m) has a selective advantage for maintaining high rates of C_4 photosynthesis, particularly when CO_2 availability is low due to restricted stomatal conductance. We measured the K_m of C_4 PEPC isozymes from 20 species in the Poaceae family and compared this data to available PEPC peptide sequences. We identified amino acid residue that are suggested to be partially responsible for the range in K_m. This was tested by mutating single amino acid residues in recombinant PEPC enzymes expressed in an *E. coli* expression system.

Objective 3. Mechanisms regulating photosynthate partitioning

- Loss of function mutants were used to show that most, if not all of the CO_2 that is released during photosynthesis originates from the oxidative pentose pathway that forms a glucose-6 phosphate (G6P) shunt around the Calvin Benson cycle. the cytosolic version of the shunt is responsible for nearly all of the CO_2 release and is also responsible for the slow-to-label pool of carbon in photosynthesizing leaves that has been observed
- Antioxidant supplementation was tested under multiple growth conditions. Media and soil growth of *A. thaliana* supplemented with archaeal antioxidants or sprayed with archaeal antioxidants grows better than controls. The same is true for *O. basilicum* grown hydroponically and on soil. The addition of antioxidants does not seem to disrupt “normal” redox signaling, because germination is normal, as is root patterning. It seems that the archaeal antioxidants are metabolized because they can be used to supply *A. thaliana* with S in media that lacks it. Further evidence that the archaeal antioxidant is effective in areal tissues is when applied through the media, it reduces the cytoplasm of leaf epidermal cells. Finally, the antioxidant seems to improve the ability of the plant to disperse excess light energy through non-photochemical quenching. An important outcome at the NC1200 meeting is a budding collaboration with Jeff Harper (NV-AES) to investigate the role of the archaeal antioxidant on Ca^{++} signalling.

- Recent work has delineated the function of two enzymes in the inositol pyrophosphate (PP-InsP) signaling pathway. The identity of the plant enzyme that converts inositol hexakisphosphate ($InsP_6$) to $InsP_7$ was delineated. This enzyme, inositol trisphosphate kinase (ITPK), was already known to catalyze $InsP_3$ to $InsP_4$ to $InsP_5$ steps in the synthesis pathway. Overexpression of ITPK yields unrestricted PP-InsP synthesis and unique phosphate accumulation properties. The VIP enzyme was also characterized and has been shown to be responsible for converting $InsP_7$ to $InsP_8$. Together this work establishes the final two steps in the PP-InsP pathway. Other studies have focused on lipid changes that occur in response to low phosphate conditions in plants with altered PP-InsP content. Our work shows that the accumulation of specific PP-InsPs greatly influences the lipid remodeling process, in response to changes in phosphate.
- The work aims to convert the secondary slow metabolism of the terpenoid biosynthetic pathway into a primary activity in model cyanobacteria, and to generate heterologous products using these photosynthetic microorganisms as cell factories. Case study is the production of the 10-carbon monoterpene β-phellandrene (PHL) in *Synechocystis* sp. PCC 6803 (*Synechocystis*). Barriers to this objective include the slow catalytic activity of the terpenoid metabolism enzymes that limits rates and yield of product synthesis and accumulation. "*Fusion constructs as protein overexpression vectors*" were applied in the overexpression of the geranyl diphosphate synthase (*GPPS*) and β-phellandrene synthase (*PHLS*) genes, causing accumulation of GPPS up to 4% and PHLS up to 10% of the total cellular protein. Such protein overexpression compensated for their slow catalytic activity and enabled transformant *Synechocystis* to constitutively generate 24 mg of PHL per g biomass (2.4% PHL:biomass, w:w), comprising improvement over earlier yields.
- We had previously demonstrated that Pho1 and the variant form Pho1ΔL80 interact with PsaC with the latter enhancing PSI activity and growth. We now show that *pho1⍰* plants expressing the Pho1ΔL80 exhibit elevated CO_2 assimilation rates compared to wildtype and *pho1⍰* plants expressing the wildtype Pho1 gene. Despite the differences in photosynthetic properties by *pho1⍰* plants expressing Pho1ΔL80 and Pho1, *in vitro* O2 electrode studies of PS1 activity showed that no differences in the stimulation by recombinant Pho1ΔL80 and Pho1.
- We have completed studies on RBP-P and RBP-L, two RNA binding proteins that recognize the prolamine and glutelin zip code targeting sequences. Both RBPs are essential for faithful RNA localization. Ongoing studies demonstrated that these two RBPs interact with the membrane trafficking factors, Rab5 and NSF, which support the role of membrane vesicular transport in mRNA transport and localization.
- Recent investigations have included repartitioning of carbon from starch to tobacco in tobacco lines that are overexpressing multiple genes and that result in levels of 30% lipid or more as a component of biomass. Other studies in the lab are describing the impact of altering C_4 photosynthetic processes, and recent transient labeling experiments are focused on 'inactive' pools and slow labeling metabolites that may be a consequence of storage pool turnover but also may result from cellular heterogeneity or just asymmetry in carbon bond rearrangements in metabolism.
- Starch is an important metabolite in both source and sink metabolism. In rice we have shown that yield increases associated with increased starch biosynthetic rates are reliant upon plant nutrition. We are examining how starch biosynthetic rates impact wheat yield. The starch regulatory pathway is fairly complex. We identified the transcription factor WRKY76 as highly upregulated in rice leaves with increased biosynthesis. We found that overexpression of WRKY76 in leaves leads to increased photosynthetic rates and plant yield. A second area of research is examining how genes that impact yield affect photosynthetic rates. The incorporation of Reduced Height (*Rht*) alleles into cereals led to yield increases in the 1970s. In wheat, all major varieties have one of two *Rht* semi-dwarfing alleles. We found that the semi-dwarfing allele *Rht-B1b* reduces flag leaf photosynthetic rates at anthesis and leads to reduction in seed size, beginning shortly after anthesis. We have identified new A, B, and D genome specific *Rht* alleles and are testing their impact on plant growth and development.

Objective 4: Developmental and Environmental Limitations to Photosynthesis.

- Recent work has laid the foundation for CAM Biodesign by analyzing the subcellular localizations and effects of overexpression of 13 enzymes and regulatory proteins of the C_4 metabolism cycle of CAM from the common ice plant in stably transformed *Arabidopsis thaliana*. Other projects explored the utility of CAM plants (e.g., *Opuntia*) for use as forage and fodder for dryland agriculture and examined diverse traits that can be leveraged for improving both water-use and nutrient-use efficiency in crops. We also leveraged genomic resources from CAM species to explore the mechanistic basis of CAM evolution, salinity tolerance, and betalain production.
- Stomatal aperture places one of the greatest limitations on carbon exchange and photosynthesis. To better understand the ancestral mechanisms of stomatal regulation in angiosperms we investigated the nature of stomatal responses in ferns and the role of leaf anatomy and hydraulics in regulating gas exchange in these early vascular plants. We found that ancestral stomatal regulation in vascular plants was a very simple process regulated by leaf hydraulics. We have also found that hydraulic function and embolism resistance in individual leaves is critical for determining leaf survival and the capacity to recover from drought stress.
- We discovered that a loss-of-function of a putative protein O-fucosyltransferase (OFT1) in *Arabidopsis* results in reduced pollen fertility and seed set. The working model is that OFT1 functions in the trans-Golgi to transfer fucose residues to proteins being secreted to the cell wall. A role for OFT1 in regulating cell wall integrity is

supported by the isolation of two suppressor mutations that are predicted to disrupt other cell wall modification pathways.

- A method was developed to evaluate pollen fitness using a reporter to quantify reactive oxygen species (dichlorodihydrofluorescein diacetate) coupled with flow cytometry and cell sorting. This technique can be used to analyze different pollen subpopulations to study the physiological responses to heat stress.
- Research continued into the phenotype of a plant harboring a triple knockout of *Arabidopsis thaliana* Ca^{2+}-ATPases 1, 2, and 7. Imaging of cytosolic Ca^{2+}dynamics triggered by light stress or a pathogen elicitor provided evidence that a loss of *aca1/2/7* results in Ca^{2+}-transients with larger magnitudes and duration, consistent with a role these Ca^{2+}-pumps in restoring basal Ca^{2+}levels after a Ca^{2+}-signal. Our results provide evidence that Ca^{2+}-pumps ACA1, 2, and 7 function in the ER and together make important contributions to Ca^{2+}- signaling dynamics in both vegetative and reproductive tissues.
- 147 commercial hybrids were grown at three sites (Yorkville, Champaign, and Harrisburg) in Illinois under three N rates (0, 60, and 280 lbs N/acre), three plant densities (32,000, 38,000 and 44,000 plants/acre), and two row arrangements (20 and 30 inches). Hybrids exhibited wide ranges in their yield responses to the different agronomic parameters tested. Narrower row spacing (20") tended to be a better arrangement of 44,000 plants/acre, and was conducive to the highest yields in the trial. Similar yield responses to narrow row spacing were observed at all locations. Understanding the agronomic factors that most influence grain yield for different corn genotypes will allow growers to most efficiently produce the greatest yields.
- Field studies identified differential response to water supply in photosynthesis, stomatal conductance and transpiration in biomass sorghum lines and a novel perennial oilseed crop. Other studies identified sorghum lines, subjected to a mutagen, with likely increased bundle sheath leakage. Using field based infrastructure and integrated with cyber-physical system we have demonstrated a degree increase in nighttime during grain filling to result in ~5% reduction in yield. In addition, we show a significant reduction in starch lead to an increase in protein and lipid accumulation in wheat grains, which can potentially lead to poor quality bread and other food products.
- To understand the molecular mechanism how silicon improves the growth of soybean plants grown under water limiting conditions, the effects of silicate application on chloroplast protein expression were examined. Intact chloroplasts were isolated from the leaves of silicon-treated and control plants subjected to water deficit stress. Proteins were then prepared from isolated chloroplasts. Two-dimensional gel electrophoresis and mass spectrometry approaches were used to identify differential chloroplast proteins in response to silicon application under water deficit stress. Proteins that shown differential expression in response to silicon application include photosynthetic proteins and enzymes. These results suggest that silicon application could affect enzymes important for photosynthesis and stabilize photosynthetic proteins and enzymes under water deficit stress.

Outputs

See Publications below.

Plans for the Coming Year:

Objective 1. Identify Strategies to optimize the assembly and function of the photosynthetic membrane.

- We will explore how FAD4 is tied into the redox state of the chloroplast.
- We will continue to study the function of a rhomboid protease located in the chloroplast envelope membranes on phosphatidic acid biosynthesis.
- We continue to investigate the identity and location of chloroplast phosphatidic acid phosphatases to come to a better understanding of phosphatidic acid metabolism in the chloroplast envelope membranes.
- Publish our work on thylakoid ion transport/channels. The data acquisition is finalized, and a manuscript is in preparation and almost finalized.
- Complement the biochemical work on cytochrome b6f redistribution by immunogold labeling of cytochrome subunits in thin sections followed by electron microscopic analysis.
- Start electron microscopic studies on thylakoid ion transporter/channel mutants for dark and light adapted plants.

Objective 2. Identify strategies to modify biochemical and regulatory factors that impact the photosynthetic capture and photorespiratory release of CO_2.

- Complete the algal omics analysis and mutant screen results, combine the datasets to identify genes and pathways that are important for heat tolerance in algae and land plants;
- Understand the regulation of NPQ and Rubisco activase in C_4 model plant *Setaria viridis*;
- Investigate the regulation of CEF in UWO241 and the model green alga *Chlamydomonas.*

- Publish research on genetic reduction of PEPC activity in *S. viridis*. We are currently preparing a manuscript on the temperature response of C_4 photosynthesis in these plants.
- Leverage parallel evolution of PEPC in the grass family to identify novel allelic variants that define key amino acid changes from a range of C_3 and C_4 species.
- Synthesized PEPC alleles are being expressed in *E. coli* to test specific amino acid substitutions on PEPC kinetic properties.

Objective 3. Mechanisms regulating photosynthate partitioning

- Use $^{13}CO_2$ flux balance analysis to better understand the cytosolic G6P shunt
- Determine if the loss of the G6P shunt eliminates the slow labeling component of the Calvin Benson cycle
- Measure plant growth, yield, and resilience of plants lacking the G6P shunt
- Determine if early age application of the antioxidant has the greatest effect.
- Radiolabel the antioxidant and see what tissues it targets and what products it makes.
- Use transcriptomics to determine the genes responding to the archaeal antioxidant.
- Try growing plants in variable lighting to see if growth effects are more pronounced.
- Use known Arabidopsis mutants to determine if the NPQ effect is in part responsible for biomass yields response to the antioxidant.
- Refine observed changes in lipid remodeling in PP-InsP mutants and transgenic plants.
- Characterize phosphate accumulation properties in PP-InsP mutants and transgenic plants.
- Engineer desired signaling and phosphate accumulation properties in pennycress.
- Engage ~300 high school students in authentic inquiry with PP-InsP mutants.
- Expand the proprietary *Fusion constructs as protein overexpression vectors* to include overexpression of additional heterologous genes in model cyanobacteria
- Compare the activity of isoprenoid biosynthetic enzymes in their native versus fusion construct formulations.
- Continue studies to determine the role of the Pho1's L80 peptide in negatively modulating photosynthetic properties.
- Initiate studies to define the RNA footprints of selected RNA binding proteins essential for RNA localization.
- Document the differences in metabolic fluxes in algal central metabolism under auto- or mixotrophic metabolism
- Quantify the metabolic fluxes through tobacco lines altered with high lipid production
- Describe changes in maize C4 photosynthesis under altered conditions
- Publish reports on transcriptome and genome sequencing of two obligate CAM species: *O. cochenillifera* (diploid) and *O. ficus-indica* (octoploid).
- Publish research into the beneficial effects of expressing multiple synthetic gene circuits of CAM C_4 metabolism cycle in *Arabidopsis thaliana* and complete the molecular, physiological, and photosynthetic analysis of selected transgenic lines.
- Publish the results of biomass productivity studies for three different *Opuntia* species and report on the causative agents of *Opuntia* stunting disease.

Objective 4: Developmental and Environmental Limitations to Photosynthesis.

- To investigate variation in embolism resistance and stomatal regulation in maize and closest relatives.
- To study the evolution of stomatal perception by the hormone abscisic acid (ABA), particularly in the gymnosperms and earliest diverging angiosperms.
- To investigate the relative roles of plant hydraulics and hormones limiting photosynthetic recovery following drought.
- Measure starch levels and gene expression of carbon and nitrogen metabolism genes in wheat varieties from the last 100 years.
- Determine how artificial selection has impacted carbon and nitrogen metabolism.
- Test candidate genes for their ability to improve heat-stress tolerance in pollen.
- Determine how Ca^{2+}signals are modified by regulation of Ca^{2+}pumps and channels.
- Investigate the role of lipid flippases in promoting cellular expansion and plant growth.
- Repeat experiment to ascertain weather-year effects.
- Investigate alternate models of hybrid characterization for yield production. Understand how hybrids differ in their response nitrogen level, plant density and row spacing
- Investigate functional relationships between canopy light utilization and spectral reflectance patterns.
- Screen Tx642 mutants for truncated light-harvesting apparatus (TLA), using remote sensing tools and protocols developed in 2018.
- Report water deficit effects on leaf photosynthetic activity and primary productivity of *Sorghum bicolor* and *Silphium integrifolium*
- Determine the role of functional stay-green as a means to reduce the negative impact on carbon balance (photosynthesis versus respiration) under high nighttime temperature
- Identify genomic regions controlling high nighttime temperature tolerance to optimize carbon balance in winter

wheat

- Publish the results of silicate application on vegetative growth and photosynthetic biomass of soybean plants grown under water limiting conditions.
- Publish the results of silicate application on protein expression of soybean plants grown under water deficit stress.

Impacts

1. Our discovery of a rhomboid protease affecting central lipid metabolism in the chloroplast opens up a new paradigm how lipid biosynthesis in chloroplasts and ultimately the assembly of the photosynthetic membrane is regulated in plants. Connecting the FAD4 activity with the redox state of the chloroplast potentially links lipid metabolism with abiotic stress responses protecting photosynthesis under adverse conditions. This work has been funded by a grant from the Chemical Sciences, Geosciences, and Biosciences Division, Office of Basic Energy Sciences, Office of Science, U.S. Department of Energy (DE–FG02–91ER20021) supporting work in the Benning lab at the MSU-DOE Plant Research Laboratory.
2. We aim to understand how photosynthetic thylakoid membranes modify their structure on different length scales (nm to microm) in response to changing environmental conditions and how these architectural alterations determine the functionality and regulation of energy conversion. This work has been funded by DOE-BES and NSF-MCB.
3. DOE-BER: Deep Green: Structural and Functional Genomic Characterization of Conserved Unannotated Green Lineage Proteins. PI Jim Umen; Co-PI: Ru Zhang, Jianlin Cheng (Mizzou), Eric Knoshaug (NREL), Ambarish Nag (NREL), Ambarish Nag (NREL);
4. DARPAR: SENTINEL: SENsing Threats In Natural Environments using Ligand-receptor modules. PI: Dimitri Nusinow; Co-PIs: Malia Gehan, Toni Kutchan, Andrea Eveland, Ru Zhang, and 7 other PIs outside of DDPSC;
5. DOE - Basic Energy Sciences - Photosynthetic Systems: Collaborative Project: Regulation of sustained Cyclic Electron Flow (CEF) in the photopsychrophile Chlamydomonas sp. UWO241. PI: Rachael Morgan-Kiss; Co-PIs: Ru Zhang, Xin Wang, Petra Fromme.
6. Our research objective is to understand how carbon fixation is limited by the leaf biochemistry and anatomy.
7. Research on auxiliary carbon pathways in photosynthesis (described in this report, conducted in the MSU-DOE Plant Research Lab)
8. research on using plants for biomaterials and bioenergy production as part of the Great Lakes Bioenergy Research Center
9. Research on common bean resilience to abiotic stress carried out as part of the MSU Plant Resilience Institute.
10. UN Systems Science: “Capturing Archaeal Biochemistry to Build Bigger Botanical Biomass.”
11. UNL Enhanced Hatch Funding, “The effects of an Archaeal Antioxidant on Photosynthesis”
12. Securing our Food: a Translational Experience for Undergraduate Students in Plant Sciences. NIFA, 01/01/2017-12/21/2020
13. Collaborative Research: Modeling the Regulatory Network of InsP6 Signaling in Plants NSF 08/15/2016-12/30/2019 (on no cost extension)
14. HHMI Inclusive Excellence. Howard Hughes Medical Institute. 05/01/2019- 08/31/2020
15. A Transdisciplinary Approach to Phosphorus Reclamation. Institute for Critical Technology and Applied Science (Virginia Tech). 07/01/19 – 06/30/21.
16. Isoprenoids, also referred to as terpenoids, are the largest class of secondary compounds in nature. More than 55,000 different molecules belonging to this family are known. Isoprenoids are useful for many diverse commercial applications, including solvents, polymers, adhesives, nature-based pharmaceuticals, nutraceuticals, flavor and fragrance compounds, as well as renewable biofuels. Outcome of the synthetic biology work in the Melis lab is to enable generation of such specialty and commodity products for human industrial and domestic consumption.
17. We have been studying the plastidial phosphorylase, Pho1, which has been found to modulate photosynthesis in addition to its role in starch biosynthesis. In a separate project, we have studying how mRNAs are sorted to distinct subdomains of the cortical endoplasmic reticulum in developing rice endosperm.
18. JJ Thelen, DK Allen, PD Bates, A Koo, D Xu; NSF/USDA-Plant Genome (PGRP): “Discovering new metabolic constraints and regulatory nodes in oilseeds engineered for enhanced fatty acid synthesis and seed oil”.
19. DK Allen, S Moose: USDA-AFRI, “Engineering C4 Photosynthesis in Maize to Enhance Nitrogen Utilization” (no cost extension).
20. Cushman JC, Yim WC, Bishop C, Claire Heinitz C; USDA-NIFA Sustainable Bioenergy and Bioproducts Challenge Area A6251. Opuntia ficus-indica: A highly water-use efficient and productive biomass feedstock for semi-arid lands. Award number: 2018-68005-27924. (05/15/18-05/14/23).
21. Investigates plant adaptation to drought stress, with a focus on stomatal regulation and embolism resistance.
22. We demonstrated that plant productivity in controlled trials can be increased by increasing leaf and seed AGPase or by selecting for specific semi-dwarfing alleles. The long-term impact of the AGPase research could be increased

yield of one or more crops under field conditions which would increase productivity and economic return for farmers.

23. The role of Ca^{2+}signaling under abiotic stress conditions (Objectives 3 and 4), and the role of lipid flippases in lipid biogenesis and catabolism pathways (Objective 3). *Arabidopsis thaliana* is being used as a model system.
24. The anticipated outcome of this project is that the knowledge gained will assist the corn breeding community to select hybrids that use solar and nutrient resources efficiently to maximize yields. Producers will be able to select corn hybrids that either are able to tolerate nitrogen- or population stress-environments, or alternatively, select hybrids that utilize greater fertilizer and other management to obtain even greater yields. The community as a whole will benefit by the corn grown with less nitrogen fertilizer runoff, thereby decreasing pollution of waterways.
25. The analysis of drought metrics as an indication of stress avoidance traits will guide development of new cultivars with enhanced drought and heat tolerance. High night temperature tolerant donors and genomic regions identified will help develop tolerant wheat cultivars with optimized carbon balance.
26. Mississippi Agricultural and Forestry Experiment Station (MAFES): “Dissecting the mechanism of a protein tyrosine phosphatase in improving drought tolerance in rice”.

Publications

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