Basic Information

Project No. and Title: NC1200: Regulation of Photosynthetic Processes

Period Covered: 11/01/2021 to 10/31/22

Date of Report: 1/18/2023

Annual Meeting Dates: 11/19/2022 virtual by ZOOM hosted by University of Nevada, Reno

Participants

Presenters:

Doug Allen (Doug.Allen@ars.usda.gov) – USDA ARS; Donald Danforth Plant Science Center; Christoph Benning (benning@msu.edu) – Michigan State University; John Cushman (jcushman@unr.edu) – University of Nevada – Reno; Katarzyna Glowack (kglowacka2@unl.edu) – University of Nebraska - Lincoln; Helmut Kirchhof (hkirchhoff@wsu.edu) – Washington State University; Jiaxu Li (jl305@bch.msstate.edu) – Mississippi State University; Tasios Melis (melis@berkeley.edu) - University of California, Berkeley; Berkely Walker (berkley@msu.edu) – Michigan State University; Ru Zhang (ruzhang.danforthcenter@gmail.com) Donald Danforth Plant Science Center;

Other Attendees: Jeffrey Harper (jfharper@unr.edu) – University of Nevada Reno; Julie Stone (jstone@unl.edu) – University of Nebraska-Lincoln; Scott McAdam (smcadam@purdue.edu) – Purdue University; Rebecca Roston (rroston@unl.edu) – University of Nebraska - Lincoln; Nicole Buan (nbuan@unl.edu) – University of Nebraska-Lincoln; Vara Prasad (vara@ksu.edu) –Kansas State University;

Brief Summary of Minutes of Annual Meeting

William Payne (Dean, College of Agriculture, Biotechnology and Natural Resources): Presented welcoming remarks to the group with an emphasis on how water resources and availability can impact agricultural productivity.

Scientific presentations (Presenters and scientific summary/goals)

Doug Allen: Reported on metabolic fluxes through photorespiration with an emphasis on the metabolism of Camelina reproductive tissues. Metabolite flux maps were described for both pods and seeds with the discovery that siliques are photosynthetically active tissues that serve as local source tissues for seeds because of their placement at the top of the canopy. However, if siliques are prevented from being photosynthetically active, seeds are still provided with about 80% of the carbon needed for seed development suggesting that Camelina has abundant and redundant source tissues for seed development.

Christoph Benning: Reported on the existence of a peroxiredoxin lipid hub involved in the sensing of redox stress within thylakoid membranes with lipids within photosystem II serving as the earliest sentinels of sensing reactive oxygen stress. The oxidation of these lipids results in signals that down-regulate the electron transport chain. Mutations in key enzymes of fatty acid desaturation and peroxiredoxin in Chlamydomonas can affect cell growth and activate key enzyme activities.

John Cushman: Reported progress on engineering synthetic versions of crassulacean acid metabolism (CAM) and tissue succulence in the C_3 photosynthetic model, Arabidopsis. A high-quality, chromosome-level genome assembly and annotation of the ice plant (*Mesembryanthemum crystallinum*) genome, which served as the source of CAM enzymes. The installation of a carboxylation gene circuit resulted in larger plants and increased nocturnal acidification, whereas the installation of a decarboxylation gene circuit resulted in plants with improved water-use efficiency and drought tolerance and decreased nocturnal acidification. Expression of both the carboxylation and decarboxylation gene circuits together resulted in plants with increased nocturnal acidification, daytime deacidification, and improved water-use efficiency and drought tolerance.

Helmut Kirchhoff: Reported on photosystem I (PS I) and PS II function and quantification of these complexes in terms of their relative abundance, associated light-harvesting complexes (LHC), and bound chlorophyll. State transitions mediated by reversible phosphorylation events were evaluated in the context of stacked and unstacked thylakoid regions. PS I and LHC can move within and outside of stacked regions of the thylakoid. Subcomplexes with PS II were also described.

Berkely Walker: Reported on photosynthesis fluxes, photorespiration, and role of photorespiration in constraining photosynthesis during light transients to optimize photorespiration using ^{13}C CO_2-labeling experiments that followed time-resolved labeling kinetics to create metabolic models. Photorespiration was shown to increase under high light, due to more CO_2 drawdown, and lower Ci. The effect of heat on photorespiration is also being examined in model plants such as Arabidopsis and Nicotiana to identify enzymes with greater thermal tolerance.

Katarzyna Glowacka: Reported on the effects that *FtsZ* mutants have on governing chloroplast size. Changing the number and size of chloroplasts within a plant cell can impact plant performance. A few large chloroplasts within a cell resulted in smaller plants, but the resulting size impairment was not large. However, plants with a few large chloroplasts showed increased excess energy absorption, improved light absorption and penetration within the leaf, and increased carotenoid accumulation.

Tasios Melis: Reported on successful strategies to use cyanobacteria as protein production factories to produce high-value recombinant fusion proteins. Successful production of proteins occurred only when expressed as fusion proteins. Fusion proteins were expressed as phycocyanin heterohexameric discs. Work on expressing the enzymes of entire biosynthetic pathways using this same strategy is in progress.

Ru Zhang: Reported on the identification of heat-tolerant mutants in *Chlamydomonas reinhardtii* and *Sertaria viridis*. Molecular bar-coded mutant collections of *Chlamydomonas reinhardtii* were used to identify mutants with impaired survival under heat stress. Approximately 50% of these mutants had no known function illustrating the potential of this approach to gain new insights into the functional weak-links associated with heat tolerance in plants.

Jiaxu Li: Reported on the proteomic analysis of the response of soybean plastids in response to silicon treatment and water-deficit stress. The interaction between silicon treatment as sodium metasilicate and drought was examined. While silicon treatment reduced the effects of water-deficit stress, the mechanistic basis of this effect is not understood.

Business Meeting Summary

NC-1200 administration. Christoph Benning remains as Administrative Advisor, but a replacement will be needed in 2025.

NC-1200 renewal. The renewal was completed in 2022 by Rob Aiken. The next renewal will be due in 2027, but work will need to be initiated in 2026.

Membership. New members are encouraged to increase membership size. Invitation of younger colleagues to participate in next year's meeting was suggested. The option of holding larger virtual meetings was discussed as a means of attracting new members.

The 2023 meeting will be held in Indiana organized by Scott McAdam.
The 2024 meeting will be held in Missouri organized by Ru Zhang.

Meeting report. Meeting report is due 60 days after the meeting by January 20, 2023.

Accomplishments

Activities in 2022 are summarized under the different objectives.

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

- The Benning lab (MI-AES) analyzed phosphatidic acid phosphatases in the chloroplast envelope membranes. LPPγ is associated with the outer envelope membrane, LPPε1 with the inner and possibly outer envelope membranes, LPPε2 is located at the inner envelope or the thylakoid membranes. The lppγε1 double mutant shows reduced growth. Acetate labeling experiments suggest that these two proteins are involved in the ER pathway of thylakoid lipid assembly but not the plastid pathway as originally proposed. A possible role for LPPε2 under dynamic conditions became apparent in a systematic Arabidopsis lipid mutant analysis for photosynthetic abnormalities. We initiated a suppressor mutant screen in the lppγε1 double mutant to identify factors responsible for the slow growth that are potentially involved in lipid signaling. (Benning and Kramer labs, MAES).
- The Benning lab (MI-AES) conducted a deletion complementation analysis of the inner chloroplast envelope rhomboid protease showing that the unusual seventh transmembrane domain is required for the RBL10 effect on lipid metabolism. Analysis of the ACP4 protein interacting with RBL10 is continuing. (Benning, MAES).
- The Benning lab (MI-AES) developed a new hypothesis for a role of FAD4/PRXQ in singlet oxygen sensing and redox regulation of photosynthetic complexes. We also identified the FAD4/PRXQ orthologs of Chlamydomonas. The FAD4 gene shares its transcriptional start and overlaps in its 5'-prime portion with the LCI2 gene, induced under low CO_2 conditions in Chlamydomonas and other green algae. The significance of this observation is under investigation (Benning and Kramer labs, MI-AES).

- The Kirchhoff lab (WA-AES) finalized and published our studies on the drought response of the photosynthetic apparatus in a resurrection plant. This study combines biochemical and in vivo spectroscopy with electron microscopy. The results indicate that the developmental state of a resurrection plant determine whether more photoprotective or more degradation-based processes are activated during dehydration.
- The Kirchhoff lab (WA-AES) reported a comprehensive study about quantification of protein supercomplexes in plant thylakoid membranes. The results show that the energy converting protein complexes are present in non-equimolar concentrations. This has far-reaching consequences on electron transport and light harvesting.
- The Kirchhoff lab (WA-AES) finalized experimental data acquisition on state transitions in plant thylakoid membranes. State transition is a reversible protein-phosphorylation dependent process that redistribute harvested light energy between the two photosystems. This ensures optimal light utilization under low light conditions. A manuscript is in preparation.
- The Kirchhoff lab (WA-AES) revealed massive light-induced reorganizations of the entire thylakoid system including changes in the number of grana stacks and their geometrical properties via electron microscopic study on thylakoid ultrastructural dynamics.
- The Kirchhoff lab (WA-AES) developed further a coarse grain computer model to simulate the protein landscapes in thylakoid membranes. The model combines electron microscopic analysis of the thylakoid ultrastructure, quantitative biology (see second point), functional analysis of photosynthetic electron transport and light harvesting, and computer-based approach to generate realistic protein ensembles used for mathematical analysis of energy migration processes in extended pigment-protein systems.
- The Roston lab (NE-AES) developed two independent visualization systems for membrane contact sites between the thylakoid and inner envelope membranes. The first system has fused thylakoid integral membrane protein Hcf106 and inner envelope integral membrane protein Tic40 to self-assembling fragments of mVenus (mVenN and mVenC). It is characterized by a uniquely punctate fluorescent signal distinct from the expression of controls with both portions of mVenus targeted at either inner envelope or thylakoid membranes. Correct targeting of the fragments is supported by chloroplast fractionation. The second system, based off the ER-plasma membrane contact site sensor “Mapper” fuses an enzymatically-dead version of SENSITIVE TO FREEZING 2 acting as an MGDG-binding protein to a rigid inner envelope anchor. Various versions of the anchoring system yield progressively more or fewer puncta. The current effort is screening conditions with these
- The Roston lab (NE-AES) completed the screening of 26 candidates for photosynthetic lipid transport within the chloroplast, with 6 more in the pipeline, and 5 candidates confirmed to be within the chloroplast.
- The Roston lab (NE-AES) developed a fractionation method that isolates a unique fraction of inner envelopes that has the density of thylakoids and a unique fraction of thylakoids that has the density of inner envelopes. Mass spectrometry revealed a protein composition not characteristic of either membrane. This fraction may represent membrane contacts between the two membranes, and we are setting up a proximity-labeling system to confirm this hypothesis.

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

- The Buan Lab (NE-AES) in collaboration with the Roston (NE-AES), Glowacka (NE-AES), and Stone labs (NE-AES) has demonstrated enhanced soybean productivity through foliar spraying of an archaeal antioxidant (AA). We continue to investigate the molecular mechanisms of AA enhancement of plant growth. Transcriptomic experiments with Arabidopsis suggest AA stimulates sialicylic acid signaling, and photosynthesis measurements with mutant strains in genes involved in non-photochemical quenching supports the interpretation that AA improves photosynthetic efficiency.

- The Buan Lab has generated separate *E. coli* expression constructs for genes to produce AA from a supplementable compound. Both enzymes for AA synthesis can be detected from a dual-expression vector, but only one of them can be detected when monocistronically expressed. The Buan Lab continues to investigate the structural stability and function of the unstable enzyme.

- The Cushman lab (NV-AES) obtained accurate vegetative and fruit biomass production data for cactus pear (*Opuntia* spp.) in the first long-term, five-year study in the United States. The effects of acute water-deficit stress on cactus pear (*O. ficus-indica*) were also investigated using metabolic profiling. The lab characterized the composition of a microbial consortium using molecular barcoding with potential for improving the biological degradation of cactus pear biomass for biofuel production. Ongoing projects also advanced progress towards the stable transformation of cactus pear (*O. ficus-indica*).

- The Cushman lab (NV-AES) reported on the potential of false flax (*Camelina sativa*) to serve as a climate-resilient biofuel feedstock within the context of the global climate crisis.

- The Walker lab (MI-AES) finished their investigations concerning amino acid transport out of the photorespiratory “cycle”, which have been accepted for publication. These investigations revealed that glycine pools vary substantially with photorespiratory rate and can affect rates of net CO_2 fixation during transients in light.

- The Walker lab (MI-AES) started investigations of the links between photorespiration and one-carbon metabolism

Objective 3. Mechanisms regulating photosynthate partitioning

- The Allen lab (MO-ARS) published a comprehensive flux map on tobacco that produce high levels of lipids in leaves. The production of lipids in vegetative tissue represents an important potential model and future crop paradigm for lipid-based biofuel production. Tobacco lines that were engineered to make more lipid, do so at the expense of non-transient starch which otherwise accumulates in the leaves. As tobacco has been domesticated for leaf biomass and less for seeds, some varieties have unique combined source and sink capacities in leaves.

- The Allen lab (MO-ARS) characterized extensively malic enzyme overexpression lines in soybean, resulting in a ~1% increase in seed oil without losses in protein levels. The subcellular location of the malic enzyme in the chloroplast is important for the enhanced lipid production and also alters fatty acid content. Enhanced malic enzyme levels that are targeted to the mitochondria and also found in the cytosol result in changes in amino acid composition indicating an increased supply of pyruvate for branched chain amino acids. A manuscript has been peer-reviewed and is under revision.

- The Allen lab (MO-ARS) developed a Camelina silique culturing system that was used to assess the metabolic fluxes of combined reproductive organs and the resulting increased carbon use efficiency that represents an emergent property of the plant. Significant carbon in the developing seed is a result of carbon assimilation in the silique wall (approx. 33-45% of total seed carbon). Leaves provide the remaining carbon for the seeds but also support other parts of the plant including roots and young developing leaves that represent other sinks. The assimilation by the silique is developmentally and spatially coordinated with the seeds and therefore may offer efficiencies such as reduced carbon translocation, or as it is a local supply of carbon may be important to the viability of a subset of seeds within the silique as discussed in the recent publication.

- The Melis Lab (CA-AES) documented the overexpression of heterologous proteins from plants, bacteria, and human, as *fusion constructs* with the abundant CpcB β-subunit of phycocyanin in cyanobacteria. Recombinant proteins accumulated up to 10% of total cell protein in the CpcB*Protein fusion form. The working hypothesis for such overexpression was that CpcB*P fusion proteins somehow accumulate in a soluble and stable form in the cytosol of the cyanobacteria, retaining the activity of the trailing heterologous "P" enzyme of interest. The present work revealed a substantially different and previously unobvious picture, with the CpcB*P proteins assembling as functional $(\alpha,\beta^*P)_3$CpcG1 phycocyanin heterohexameric discs, where α is the CpcA α-subunit of phycocyanin, β*P is the CpcB*P fusion protein, asterisk denotes fusion, and CpcG1 is the 28.9 kDa phycocyanin proximal disc linker polypeptide CpcG1.

- The Melis Lab (CA-AES) further demonstrated that the $(\alpha,\beta^*P)_3$CpcG1 heterohexameric modified phycocyanin discs were functionally attached to the *Synechocystis* allophycocyanin core cylinders and, through the α,β constituents, efficiently absorb light and transfer the excitation energy from the assembled $(\alpha,\beta^*P)_3$CpcG1 heterohexamers to the PSII reaction centers, enhancing the rate of photochemical charge separation and electron transfer activity in this photosystem, thereby performing an essential cellular function.

- The Melis Lab (CA-AES) showed that the $(\alpha,\beta^*P)_3$CpcG1 heterohexameric structure was recognized by the cells as a native functional and essential feature, explaining why and how the cell tolerates the presence of the associated recombinant proteins and enables their substantial accumulation.

- The Okita lab (WA-AES) conducted further studies to establish a relationship between the plastidic starch phosphorylase (Pho1) and PsaC, the terminal electron acceptor-donor of photosystem I. Results from yeast 2-hybrid studies showed that PsaC interacts with Pho1, and various variant forms of Pho1. This interaction between Pho1 and PsaC was confirmed by bimolecular fluorescence

complementation (BiFC) studies, although the interaction was weak.

- The Okita lab (WA-AES) showed that Pho1 interacts with PsaD, a second component of photosystem I, as viewed by yeast 2-hybrid. However, such interaction was not detected by BiFC.

- The Okita lab (WA-AES) conducted further studies on L80, a negative regulatory 80 residue peptide that is absent in the human and yeast phosphorylase. To identify the proximate location of the negative regulatory sequences, transgenic rice lines harboring selective deletions of the L80 peptide sequences were generated and seeds from T1 plants collected.

- In a collaborative arrangement with the Okita laboratory (WA-AES), Corteva scientists have generated several maize lines where the L80 peptide has been removed from the maize Pho1 gene by gene editing. The Okita lab expects to soon receive kernels from these gene-edited lines for evaluation.

- The Okita laboratory (WA-AES) generated rice plants expressing CRISPR-Cas9 plasmids to edit the L80 sequences of the rice Pho1. Several rice harboring L80 deletions have been identified.

- The Roston lab (NE-AES) summarized that diverse chemical antioxidants (e.g., glutathione, melatonin, flavonoids) can be applied in diverse ways (e.g., seed coating, drenching, spraying of above-ground tissue, or stem injection) and many of these enhance growth through effects on photosynthesis in a variety of plants (Arabidopsis, soybean, maize, wheat, sorghum, etc.).

- The Roston lab (NE-AES) continued to work with an Archaeal antioxidant (ArA) to better understand the effects of applied reductants on photosynthesis. When applied by spraying on soybean, Arabidopsis, basil, and hemp, ArA enhances growth and yield and reduces non-photochemical quenching in soybeans. Using an automated, high-throughput Lemnatec 3D plant phenotyping facility we have collected high-resolution data on the impact of ArA on soybean.

- The Stone Lab (NE-AES) in collaboration with the Roston (NE-AES), Glowacka (NE-AES), and Buan labs (NE-AES) - collectively the NE Archaeal Antioxidant Power Team - has shown that foliar spraying of an archaeal antioxidant (ArA) can enhance plant growth. The molecular mechanisms of ArA enhancement of plant growth continue to be investigated.

- The Stone Lab (NE-AES) showed that photosynthesis measurements with mutant strains in genes involved in non-photochemical quenching supports the notion that AA improves photosynthetic efficiency to support enhanced growth.

- The Stone Lab (NE-AES) conducted experiments collecting Arabidopsis RNA-seq transcriptomic data, phytohormone profiling, and with mutant and transgenic lines suggesting that ArA stimulated salicylic acid signaling in conjunction with growth enhancement. These observations raise the question of whether ArA application influences plant pathogen resistance. This will be pursued in the Stone Lab.
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Objective 4: Developmental and Environmental Limitations to Photosynthesis

- The Below lab (IL – AES) obtained accurate data detailing the effects of various application methods of mycorrhizal fungi at planting of maze (*Zea mays* L.) with or without starter fertilizer on soil mycorrhizal populations, grain yield, and yield components.

- The Below lab (IL – AES) obtained accurate data of soil composition, plant growth, yield, and components on the first year of the multi-year fertilizer trial. The second-year trial using soybean [*Glycine max* (L.) Merr.] on the static plots were established, grown, and resulting grain production data was obtained.

- The Below lab (IL – AES) harvested the maize grain, made measurements, and analyzed the data resulting from adding humic acid to the early fertilization scheme for maize plants.

- The Below lab (IL – AES) characterized the interactions of multiple agronomic management techniques on soil microbiota and yield of long – term continuous maize.

- The Cushman lab (NV-AES) completed a high-quality genome assembly and detailed transcriptome analysis of the common ice plant (*Mesembryanthemum crystallinum*), to understand the genetic and regulatory requirements underpinning facultative crassulacean acid metabolism (CAM). This information was leveraged to characterize the ice plant membrane proteomes of multiple subcellular compartments and the effects of salinity stress on gene expression changes in this halophytic model.

- The Cushman lab (NV-AES) continued their phenotypic characterization of Teff (*Eragrostis tef),* a C_4 tropical grass including grain mineral nutrient profiling and iron bioavailability of selected accessions of the national Teff germplasm collection.

- The Fritschi lab (MO-AES) phenotyped a soybean diversity panel consisting of approximately 200 genotypes for leaf gas exchange rates. The soybean panel was grown in two field environment and leaf gas exchange was quantified for three replications for all entries for each environment. Net photosynthesis, ci/ca, gs, and WUEi data were extracted and genome wide association mapping was conducted. Significant markers for each of the traits were identified.

- The Fritschi lab (MO-AES) characterized carbon isotope discrimination in biparental populations and diversity panels and explored the relationship between the marker trait associations for carbon isotope discrimination and the leaf gas exchange traits mapped (previous bullet). A significant number of leaf gas exchange related markers collocalilzed with markers identified for carbon isotope discrimination. Ongoing work is aimed at identifying candidate genes in the regions identified by genetic mapping.

- The Fritschi lab (MO-AES) assessed leaf gas exchange of soybean lines contrasting in carbon isotope discrimination, canopy wilting, or canopy temperature under well-wattered and water stress conditions. Data analysis is ongoing.

- The Glowacka Lab (NE-AES) generated the non-segregating T2 generation of transgenic lines of soybean with modified non-photochemical quenching (NPQ) with the aim to reduce stomatal opening under not favorable conditions.

- The Glowacka Lab (NE-AES) selected three soybean transgenic events based on the primary NPQ results to test in the field for growth under drought conditions.

- The Glowacka Lab (NE-AES) performed a successful field experiment with selected three transgenic events of soybean under rain-fed conditions of the field.

- The Glowacka Lab (NE-AES) performed a half-season harvest of soybean transgenic lines and estimated growth differences with wild-type based on leaf area, height and dry weight of above-ground biomass. The preliminary results strongly suggest that tested genetic modification gives field-grown plants a growth advantage under water-limited conditions.

- The Glowacka Lab (NE-AES) performed an end-season harvest of soybean transgenic lines to estimate the number and weight of the seeds.

- The Harper lab (NV-AES) developed a new highly sensitive ratiometric Calcium reporter that can detect cytosolic calcium signals in response to a heat stress in leaves.

- The Harper lab (NV-AES) discovered that pollen, in contrast to leaves, failed to show a heat stress triggered calcium transient.

- The Harper lab (NV-AES) tested a strategy to improve heat stress tolerance in pollen by overexpressing a gene to increase the biosynthesis of Vitamin C (*Vitamin C Defective 2*). In contrast to reports that VTC2 overexpression improves stress tolerance in vegetative tissues, overexpression specifically in pollen rendered Arabidopsis near sterile. The negative impacts on pollen include an increase frequency of bursting and change in in growth associated Ca^{2+} signaling.

- The Li lab (MS-AES) reported that silicate application can improve the vegetative growth and photosynthetic biomass of soybean plants grown under water limiting conditions.

- The Li lab (MS-AES) reported proteomic analysis of leaf proteins in rice plants under ultraviolet-B radiation stress.

- The Li lab (MS-AES) completed proteomic analysis of chloroplast proteins in soybean plants under drought.

- The McAdam lab (IN-AES) determined that evolution into an aquatic environment can dramatically alter the function of stomata between closely related genera. Aquatic environments offer both an opportunity for the evolution of novel stomatal-regulatory traits, such as an ability to open to

extremely wide apertures increasing photosynthetic rates, or the loss of stomatal function such that water use is never regulated.

- The McAdam lab (IN-AES) determined that a simple anatomical trait of the xylem, the relative area of vessels, correlates with the embolism resistance, or drought tolerance, of species. This result has implications for predicting drought tolerance from a simple anatomical trait of the xylem.

- The McAdam lab (IN-AES) found that a very high level of the drought hormone abscisic acid, which closes stomata, is synthesized when cells lose turgor and that an interaction between the cell membrane and wall is critical for functional ABA biosynthesis. The McAdam lab also found that during a lethal drought the level of this hormone abruptly increased when leaves died.

- The Prasad lab (KS-AES) showed that high night temperature (HNT) had significant negative effect on grain macro- and micro-nutrient content. However, starch and protein concentrations were differentially correlated with grain nutrients, with starch negatively correlated with many of the micronutrients under control and HNT.

- The Prasad lab (KS-AES) determined that improvements in kernel weight (KW) have been predominantly related to an extended kernel-filling duration through the phenotypic characterization of maize (corn) hybrids from different timelines. However, the trade-off between kernel number and KW poses a challenge for future yield progress.

- The Prasad lab (KS-AES) identified that modern plants had improved post-silking N partitioning to leaves. Greater N allocation to leaves resulted in an increased post-silking carbon fixation. Internal translocation to the grains was improved due to larger supply from leaves.

- The Zhang lab (MO-ARS) employed systems-wide approaches to investigate how the model green alga *Chlamydomonas reinhardtii* responded to moderate and acute high temperatures of 35^{o}C and 40^{o}C, respectively. A paper about this work was published on Communications Biology. This work helps identify engineering targets to improve thermotolerance in photosynthetic cells.

- The Zhang lab (MO-ARS) investigated the dynamics of heat-induced cyclic electron flow (CEF) around photosystem I (PSI) in the model green alga *Chlamydomonas reinhardtii* under moderate and acute high temperatures.

- The Zhang lab (MO-ARS) completed the genome-wide pooled screens of *Chlamydomonas reinhardtii* mutants under moderate and acute high temperatures of 35^{o}C and 40^{o}C and identified a list of high confidence genes with potential roles in heat tolerance (HTGs) by triangulating HTGs with heat-induced transcripts/proteins in wildtype cultures and MapMan functional annotation data. A manuscript about this work is under revision.

- The Zhang lab (MO-ARS) generated CRISPR *Chlamydomonas reinhardtii* mutants in some select HTGs and employed Arabidopsis mutants deficient in the homologous HTGs for detailed function analysis. The information gained from the model green alga *Chlamydomonas reinhardtii* can be transformed into land plants to improve crop thermotolerance.

- The Zhang lab (MO-ARS) investigated how organic carbon source affected the responses of *Chlamydomonas reinhardtii* to moderate and acute high temperatures and the importance of carbon metabolism in thermotolerance of photosynthetic cells. A manuscript about this work was submitted and under review.

- In collaboration with the Umen lab, the Zhang lab (MO-ARS) generated a CRISPR mutant library to study unannotated and conserved green lineage proteins (called Deep Green proteins) in *Chlamydomonas reinhardtii*.

- The Zhang lab (MO-ARS) generated transgenic *Setaria viridis* mutants with altered photoprotection (non-photochemical quenching, NPQ) and enhanced photosynthesis. These results help us understand the regulation of C_4 photosynthesis and provide insights for improving photosynthesis in C_4 photosynthesis crops.

Outputs

See Publications, below.

Plans for the coming year

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

- The Benning lab (MI-AES) will complete the analysis of chloroplast phosphatidic acid phosphatases and conduct an conduct and suppressor mutant screen in the lppγε1 double mutant (Benning, MAES).
- The Benning lab (MI-AES) will complete the functional interaction of ACP4 and RBL10 (Benning, MAES)
- The Benning lab (MI-AES) will test the hypothesis on the role of FAD4/PRXQ in redox regulation in plants and algae (Benning, MAES).
- The Kirschhoff lab (WA-AES) will finalize the project on thylakoid ultrastructural dynamics by extending studies to mutants that are likely impacted in these architectural changes.
- The Kirschhoff lab (WA-AES) will finalize and publish a manuscript on state transitions.
- The Kirschhoff lab (WA-AES) will extend ultrastructural, composition, and functional studies on thylakoid reorganizations under high-energy quenching (qE) conditions.
- The Kirschhoff lab (WA-AES) will further develop dynamic coarse-grain computer models of thylakoid membranes to understand structure-function relationship for electron transport and light harvesting.
- The Roston lab (NE-AES) will use proximity labeling to test if the fractionated membranes represent membrane contacts.

- The Roston lab (NE-AES) will continue to screen candidates for photosynthetic lipid transport within the chloroplast.
- The Roston lab (NE-AES) will obtain mutants in Arabidopsis for chloroplast-located lipid transporters that we will begin to characterize, first with physiological measurements and then with measurements of lipid transport.
- The Roston lab (NE-AES) will use mutants of non-photochemical quenching and investigate the timing and location of the effects to help us understand the relevance of externally applied antioxidants to photosynthetic improvement.

Objective 2. Identify strategies to modify biochemical and regulatory factors that impact the photosynthetic capture and photorespiratory release of CO2.

- The Stone Lab (NE-AES) and the NE Archaeal Antioxidant Power Team (NE-AES) expects to publish manuscripts related to the exogenous ArA application on Arabidopsis and soybean.
- The Stone Lab (NE-AES) will test ArA-treated Arabidopsis for plant pathogen resistance. Bacterial pathogens (virulent and avirulent strains of *Psuedomonas syringae* pv. *maculicaula*) will be tested initially. Depending on the outcome(s), necrotrophic fungal pathogen and insect feeding may also be tested.
- The Stone Lab (NE-AES) will collaborate with the Roston Lab (NE-AES) to generate transgenic Arabidopsis with constructs containing genes responsible for ArA synthesis cloned in the Buan Lab (NE-AES). If promising, we will collaborate with the UNL Plant Transformation Core Facility to introduce these plant vectors for ArA biosynthesis into Arabidopsis.
- The Walker lab (MI-AES) will continue investigating the role of glycine pools under light transients in wild-type plants and mutants with increased glycine contents
- The Walker lab (MI-AES) will finish analyzing flux data determining the links between photorespiration and one-carbon metabolism

Objective 3. Mechanisms regulating photosynthate partitioning

- The Allen lab (MO-ARS) will study changes in acyl carrier protein and acyl CoA levels in developing seeds and leaves, in some instances using isotopes and with refinements to methodologies to better articulate lipid metabolism under stress.
- The Buan Lab (NE-AES) expects to publish manuscripts and file patents describing synthesis of archaeal antioxidant by *E. coli* and use of archaeal antioxidant to enhance crop productivity.
- The Buan Lab (NE-AES) will continue the structure and function of an uncharacterized enzyme involved in AA biosynthesis.
- The Buan Lab (NE-AES) will continue to work on creating a second synthetic pathway for AA biosynthesis in *E.coli* from a metabolite that is highly abundant in plant cells.
- The Buan Lab (NE-AES) will collaborate with the Roston Lab (NE-AES) and the UNL Plant Transformation Core Facility to produce plant vectors for AA biosynthesis.
- The Buan Lab (NE-AES) will explore ways to increase synthesis of AA in *E. coli* for application to plants as an organic non-toxic microbial extract.
- The Buan Lab (NE-AES) will pursue designing an in vivo biosensor for AA in collaboration with Los Alamos National Laboratory.
- The Cushman lab (NV-AES) will continue work on transcriptome and genome sequencing of two obligate CAM species: *O. cochenillifera* (diploid) and *O. ficus-indica* (octoploid).

- The Cushman lab (NV-AES) will continue its characterization of biomass productivity from field trials of 14 different elite accessions of *O. ficus-indica* and *O. cochenillifera*. The lab will also report on cladode area index (CAI) models for multiple accessions of *Opuntia*, life cycle assessment (LCA) and life cycle costing (LCC) analyses related to bioenergy production. Also, the lab will continue investigations into the causative agents of *Opuntia* stunting disease and molecular basis of spine and glochid formation.
- The Melis Lab (CA-AES) will expand its promising phycocyanin fusion constructs investigation to include, in addition to the CpcB β-subunit of phycocyanin, the CpcA α-subunit of phycocyanin, and their linker protein, the CpcG1 gene product, as carriers or independent recombinant proteins, in essence making α*P β*P, and CpcG1*P fusions with different recombinant enzymes. The aim is to over-express sequential enzymes of a biosynthetic pathway, which will be close to each other, thereby enhancing photosynthate partitioning and flux toward the desired heterologous product synthesis and accumulation.
- The Melis Lab (CA-AES) will demonstrate β-phellandrene rate and yield enhancement in cyanobacteria upon the simultaneous overexpression of the β-phellandrene synthase (PHLS), geranyl diphosphate synthase (GPPS) and Isopentenyl-diphosphate δ-isomerase (Ipi) as CpcB*PHLS, CpcA*GPPS, and CpcG1*Ipi fusion constructs. The hypothesis to be tested is whether this arrangement enhances endogenous isoprenoid biosynthetic pathway substrate flux toward the heterologous β-phellandrene synthesis and accumulation.
- The Okita laboratory (WA-AES) will continue studies to characterize the interaction of Pho1 with PsaC and PsaD. Bacterial strains harboring expression plasmids for these proteins have been constructed and currently evaluated for co-expression and protein assembly. Alternatively, pull-down studies will be conducted where immobilized recombinant proteins will be incubated with rice extracts and the captured proteins identified by immunoblotting.
- The Okita laboratory (WA-AES) will continue studies on the negative regulatory L80 peptide by evaluating the growth and photosynthetic properties of rice plants harboring various deletions of the L80 sequences. Similar studies will be conducted with the gene-edited maize plants.
- The Roston lab (NE-AES) perform an in-depth analysis of the phenotyping data on soybean to determine the impacts of applied reductants on stomatal closure and chlorophyll fluorescence. A field trial will also be conducted.

Objective 4: Developmental and Environmental Limitations to Photosynthesis

- The Below lab (IL – AES) will establish and conduct the third year of the multi – year fertilization study. Maize will be grown with a portion newly receiving every – other year fertilization treatment.
- The Below lab (IL – AES) will continue to investigate the potential of using mycorrhizal fungi to replace some fertilizer in a maize-soybean production system.
- The Below lab (IL – AES) will characterize the interactions of multiple agronomic management techniques on soil microbiota and yield of long – term continuous maize.
- The Below lab (IL – AES) plans to further investigate agronomic management of the stover refuse from growing continuous maize on long – term soil characteristics.
- The Cushman lab (NV-AES) will continue its work on optimizing synthetic CAM potentially co-engineered with tissue succulence in *A. thaliana and Glycine max*.
- The Cushman lab (NV-AES) will continue its characterization of the phenotypic diversity within the USDA-ARS germplasm collection of Teff (*Eragrostis tef*) and genome and transcriptome analysis of drought-tolerant accessions of *E. tef*.

- The Fritschi lab (MO-AES) will continue work on genetic mapping of soybean leaf and leaf photosynthesis traits. Data extraction from the past phenotyping campaign will continue and image-based leaf analysis will be conducted to relate gas exchange data to leaf characteristics.
- The Fritschi lab (MO-AES) will follow up on photosynthesis responses in lines contrasting for carbon isotope discrimination, canopy wilting, and canopy temperature as influenced by water availability. Experiments will be conducted under field conditions, leveraging rainout shelters to control the level of water input.
- The Glowacka Lab (NE-AES) will continue to characterize seed yield from the 2022 field-grown transgenic soybean with NPQ modification.
- The Glowacka Lab (NE-AES) will lead to homozygosity the new upcoming soybean transgenic events with modified NPQ.
- The Glowacka Lab (NE-AES) will test for the effectiveness of varied promoters in the modification of stoma behavior through NPQ in soybean.
- The Glowacka Lab (NE-AES) will continue to characterize soybean transgenics with modified NPQ for physiological phenotype under control and stress conditions.
- The Glowacka Lab (NE-AES) will repeat the field experiment from 2023 soybean transgenics with modified NPQ to confirm the results obtained in 2022.
- The Harper lab (NV-AES) will test candidate genes for their ability to improve heat-stress tolerance in pollen.
- The Harper lab (NV-AES) will determine how Ca^{2+}signals are modified by regulation of Ca^{2+}pumps and channels.
- The Harper lab (NV-AES) will investigate the role of lipid flippases in regulating heat-stress tolerance.
- The Li lab (MS-AES) will work on publishing the results of silicate application on protein expression of soybean plants grown under water deficit stress.
- The Li lab (MS-AES) will work on peptidomic analysis of small polypeptides involved in regulating drought stress response in rice.
- The McAdam lab (IN-AES) plans to conduct experiments to determine when stomatal function begins during leaf expansion, to determine when plant gas exchange can be regulated.
- The McAdam lab (IN-AES) plans to determine the specific signals driving stomatal closure during drought, particularly the drives of water potential decline during drought.
- The Prasad lab (KS-AES) will conduct experiments to understand impacts of temperature response of key grain crops on emergence, vegetative growth, reproductive physiology and yield under controlled and field environments.
- The Prasad lab (KS-AES) will quantify the impact of high temperature on grain quality traits for food crops from field and controlled environmental conditions.
- The Prasad lab (KS-AES) will quantify the response of weeds and herbicide efficacy under different environmental conditions (e.g., temperatures).
- The Zhang lab (MO-ARS) will publish about the genome-wide pooled screens of *Chlamydomonas reinhardtii* under high temperatures and the list of high confidence genes with potential roles in heat tolerance (HTGs).
- The Zhang lab (MO-ARS) characterize functions of select heat tolerance genes (HTGs) in Chlamydomonas and Arabidopsis.
- The Zhang lab (MO-ARS) will investigate the regulation and function of heat-induced cyclic electron flow (CEF) in the model green alga *Chlamydomonas reinhardtii*.
- The Zhang lab (MO-ARS) will understand the regulation of NPQ in the C_4 model plant *Setaria viridis* and publish the related paper.

- The Zhang lab (MO-ARS) will characterize Deep Green proteins in *Chlamydomonas reinhardtii* using high-throughput functional genomic approaches and quantitative pooled screens under different conditions.

Impacts

Plant photosynthesis is essential for the production of fuels, food, feed, and fiber used to sustain livestock and human life. Therefore, this multistate project has resulted in many key impacts relevant to these topics. A few example impacts are summarized below.

Understanding partitioning of carbon involves central metabolism, possibly the most well-documented set of pathways; however, central metabolism is flexible and context specific, differing in species, tissues and responding to inputs from environment.

Significant progress was made in characterizing the soil microbiota associated with growing maize continuously long – term. This information provides a basis for producers to grow continuous maize more sustainably based on their field soil type.

Yield responses in maize production were demonstrated using various application methods, mycorrhizal fungi inoculants, fertilization regimes, including applying humic acid.

The discovery of a chloroplast rhomboid protease affecting central lipid metabolism and chloroplast phosphatidic acid lipases affecting growth opens up a new paradigm how lipid biosynthesis in chloroplasts and ultimately the assembly of the photosynthetic membrane is regulated.

Connecting the FAD4/PRXQ activity with the redox state of the chloroplast potentially links lipid metabolism and lipid signaling with abiotic stress responses proving new approaches for protecting plant photosynthesis and productivity under adverse conditions.

An archaeal antioxidant was discovered and characterized that when supplied to plants in media or as a foliar spray increases photosynthetic efficiency resulting in faster growth and higher crop yields.

Progress towards understanding the genetic and regulatory requirements of facultative CAM was made through the sequencing of the ice plant genome that will inform ongoing and future efforts to engineer crassulacean acid metabolism (CAM) into both model (*A. thaliana*) and crop (*G. max*) species.

Progress was made towards engineering synthetic CAM to improve water-use efficiency under harsh environmental conditions and carboxylation module engineering for enhanced photosynthetic capture over the diel cycle to improve plant productivity.

The phenotypic diversity within the USDA-ARS germplasm collection of Teff (*Eragrostis tef*) was characterized, which included the identification of drought-tolerant accessions of *E. tef* and detailed investigations into the mechanistic basis of their drought tolerance.

Genetic markers were identified for carbon isotope discrimination and leaf gas exchange traits and leveraged to develop more drought tolerant germplasm.

Progress was made on the comparative physiological characterization of photosynthetic responses in soybean genotypes contrasting in canopy temperature, canopy wilting, and carbon isotope discrimination in response to water deficit stress.

A cytosolic calcium signal was identified as one of the earliest known responses in the leaf to a heat stress. Insights into how plants sense and respond to heat are expected to guide future efforts to engineer plants

to be more productive under heat stress conditions.

Significant differences were identified in how pollen and vegetative cells sense and respond to heat stress, which suggests that strategies to improve heat stress tolerance in whole plants might not be successful in the context of plant reproduction (i.e., we need to find pollen specific strategies to improve reproductive stress tolerance).

Progress was towards understanding the ultrastructural dynamics under short-term (<1 hour) low light conditions, which increased our understanding of how plants adapt to fluctuating sunlight regimes as realized under field conditions.

Tools were developed for the accurate protein quantification, which are essential for computer simulations of photosynthetic light reactions. Computer simulations are a central tool to understand the often-non-intuitive behavior of the energy converting machinery in thylakoid membranes that can be employed for intelligent design efforts of photosynthesis.

An experimental platform was developed for studying thylakoid membrane dynamics on intact leaf tissues with electron microscopy to provide insights into the dynamics of structure-function relationship of the photosynthetic apparatus with high spatial resolution.

The application of silicate improves the vegetative growth and photosynthetic biomass of soybean plants grown under water limiting conditions. Thus, silicate supplementation may serve as a promising strategy for improving soybean growth and photosynthetic productivity of soybean plants grown under drought condition.

UV-B responsive proteins were identified in leaves of rice plants providing new insights into understanding how rice plants are tailored to UV-B stress via modulating the expression of UV-B responsive proteins.

Detailed investigations of anatomical traits, ABA biosynthesis, and stomatal-regulatory traits provided new insights into the mechanistic basis of drought tolerance and stomatal regulation during drought.

Experiments revealed that the selection of a strong promoter to express the desired recombinant protein in eukaryotic systems does not necessarily translate into substantial amounts of the target protein in photosynthetic systems.

Methods for overexpressing eukaryotic proteins heterologously to levels up to 10% of the total cellular protein were achieved for plant terpene synthases, human interferon, and the bacterial tetanus toxin fragment C in photosynthetic cyanobacteria.

The starch enzyme Pho1 was shown to interacts with PsaC. This interaction is likely responsible for the observed changes in photosystem 1 properties exhibited by rice plants expressing a Pho1 variant lacking the negative regulatory L80 peptide (Pho1ΔL80).

Significant progress was made in determining whether gene edited maize lines expressing Pho1ΔL80 possess faster growth rates, enhanced productivity and grain yields, and increase photosynthetic capacity.

A negative correlation between high temperature and grain quality highlighted the imperative balance of seed micronutrient composition that needs to be maintained as efforts are intensified to enhance grain yield under favorable and warming environments.

Different genotypes/hybrids of maize were identified and trends and current state of development helped to identify candidate targets and opportunities for future improvements.

A better understanding of responses of crop and weeds to environmental factors (particularly high temperature stress) and herbicide-use efficiency resulted in improved strategies for weed management and developing herbicide resistance.

Photorespiration, a pathway related to photosynthesis that response directly to increased CO_2 and temperature, was linked to key metabolic pathways that are essential for human nutrition (amino acid synthesis and one-carbon metabolism).

Improved understanding of the regulation of photosynthesis under abiotic stresses (heat) in both green algae and land plants have identified potential targets to improve stress tolerance in photosynthetic organisms.

In addition to these research discoveries, salary support assisted in the development of multiple research proposals at the federal, local, and industrial partnership levels on photosynthesis, many successful. Our regular meetings enhanced collaborative efforts among many members of the group. The resulting projects have trained and employed many early career scientists, improving capacity for photosynthetic research in the future while simultaneously moving forward the current state of knowledge.

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