## Basic Information

- **Project No. and Title:** NC1200: Regulation of Photosynthetic Processes
- **Period Covered:** 11/01/2024 to 10/31/2025
- **Date of Report:** 11/01/2025
- **Annual Meeting Dates:** 10/18/2025

## Participants

Benning, Christoph (benning@msu.edu) - Michigan State University; Cushman, John (jcushman@unr.edu) – University of Nevada Reno; Cousins, Asaph (acousins@wsu.edu) – Washington State University; Gillaspy, Glenda (glenda.gillaspy@wisc.edu) – University Wisconsin; Glowacka, Katarzyna (kglowacka2@unl.edu) – University of Nebraska; Harper, Jeff (jfharper@unr.edu) – University of Nevada Reno; Jiang, Nan (nanjiang@hawaii.edu) – University of Hawaii; Melis, Anastasios (melis@berkeley.edu) – University of California, Berkeley; Tony Studer (astuder@illinois.edu) – University of Illinois; Roston, Rebecca (rroston@unl.edu) – University of Nebraska; Zhang, Ru (rzhang@danforthcenter.org) – Donald Danforth Plant Science Center; Wang, Xin (wangx3@ufl.edu) – University of Florida

Note that Benning and Gillaspy participated in only the business meeting and not in the presentations and research sessions.

## Brief Summary of Minutes of Annual Meeting

The meeting was held at Washington State University in Vancouver, Washington. Helmut Kirchhoff and Asaph Cousins (WSU) began the meeting with introductions and discussed the report format and completion.

**John Cushman,** NV-AES reported that tissue succulence engineering in the model Arabidopsis and the crop soybean (*Glycine max*) improved water-deficit (drought) tolerance through a combination of increased water availability within leaves as measured by increased leaf thickness and leaf succulence and reductions in rates of leaf water loss through transpiration with decreased stomatal density, stomatal aperture, and stomatal conductance that resulted in increased instantaneous water-use efficiency. Acute water-deficit stress tests confirmed that expression of a basic helix-loop-helix transcription factor from winegrape under the control of a strong constitutive promoter resulted in >95% survival compared with an 8% survival rate of control lines. Tissue succulence engineering also increased flower, pod, and seed size that translated into a 28% increase in overall seed yield under unstressed conditions.

**Asaph Cousins**, WA-AES indicated 500 water molecules are lost for every CO2 coming in which results in significant water loss to maintain transpiration stream. Bicarbonate and PEP levels will impact PEPC activity, and bicarbonate is itself affected by carbonic anhydrase. Additionally, how much PEPC or its affinity may play a role as indicated in Flaveria. The goal is to look at factors causing low intracellular CO2 that might be causing a loss in C4 photosynthesis. [HCO3] levels are in part controlled by carbonic anhydrase. The Cousins lab is looking for other ways to shift the curve and influence C4 efficiency.

**Mike Giroux**, MT-AES reported on a project investigating the relationship between wheat productivity, photosynthetic performance, and starch metabolism. Leaf starch content was found to correlate negatively with flowering time, plant biomass, and seed yield. QTL mapping identified a locus on chromosome 3D associated with leaf starch, flowering time, and yield. Ongoing fine mapping and candidate gene analyses aim to pinpoint the gene underlying this QTL. Once identified, gene specific markers will be developed to support selection for improved plant productivity. The overall goal is to advance breeding strategies that enhance photosynthesis, starch utilization, and crop performance.

**Katarzyna Glowacka,** NE-AES obtained evidence for a dark accumulation of the zeaxanthin in high chilling tolerance C4 grasses of Miscanthus. Chilling-induced zeaxanthin accumulation in the dark enhanced rate of NPQ induction by 66% in the following morning. The possible mechanisms uncovered here for the unique regulation of NPQ include post-translational regulation of violaxanthin de-epoxidase (VDE), VDE cofactor accessibility, and absence of transcriptional upregulation of zeaxanthin conversion back to violaxanthin. Engineering dark accumulation of zeaxanthin will help improve crop chilling tolerance and promote sustainable production by allowing early spring planting to maximize the use of early-season soil moisture.

**Jeff Harper**, NV-ARS reported progress on understanding the connection between a disruption of four glucosyl ceramidase (GCD) genes and the development of a salicylic acid (SA)-dependent autoimmunity phenotype. Before the onset of any visible lesions, leaf cells in the quadruple GCD knockout plants showed an increase in their basal cytosolic $Ca^{2+}$ ($[Ca^{2+}]_{cyt}$). The introduction of a CRISPR/Cas9 edited knockout of a calcium dependent protein kinase (CPK) resulted in a partial suppression of the autoimmunity phenotype. Together these results support a model in which an increase in basal $[Ca^{2+}]_{cyt}$ can trigger programmed cell death through a pathway involving a $Ca^{2+}$ regulated CPK.

**Helmut Kirchhoff**, WA-AES reported approximately 70% stimulation of photosynthetic electron transport rates in Arabidopsis thylakoid membranes through a simple preillumination protocol. Comparing different mutants, we can rule out that this significant boost is caused by protein phosphorylation, thylakoid ion channels, changes in the proton motive force, or cyclic electron transport around photosystem I. Detailed spectroscopic analysis indicates that the acceleration of electron transport is likely due to improved mobility of the small electron carrier plastoquinone, which connects photosystem II with the cytochrome b6f complex. This is supported by fluorescence recovery after photobleaching measurements. We suggest that enhanced

plastoquinone diffusion results from light-induced reorganization of the protein landscape within thylakoid membranes.

**Tasios Melis**, CA-AES reported on converting fast-growth unicellular cyanobacteria into cell factories for the renewable and carbon-negative generation of high-value compounds and biopharmaceutical proteins. In both cases, there is a need to alter the endogenous photosynthetic carbon partitioning so as to arrive at high yields of the exogenous product. Cyanobacteria are prokaryotic photosynthetic microorganisms that can generate, in addition to biomass, useful chemicals and proteins / enzymes, essentially from sunlight, carbon dioxide and water. Selected aspects of cyanobacterial production (isoprenoids and high-value proteins / enzymes), and scale-up methods suitable for product generation and downstream processing were addressed in this period. The work focused on the promise of specialty chemicals and proteins production, with isoprenoid products and biopharma proteins as study cases, and the challenges encountered in the expression of recombinant proteins / enzymes, which underline the essence of synthetic biology with these photosynthetic microorganisms. Progress and the current state of the art in these targeted topics were reported.

**Rebecca Roston**, NE-AES reported on how exogenous antioxidant treatments influence photosynthetic photoprotection and growth in Arabidopsis thaliana. Building on prior work showing CoM enhances plant vigor (Brown et al., Antioxidants 2025), we tested six common antioxidants in parallel using a high-throughput chlorophyll-fluorescence system. Antioxidant application differentially affected non-photochemical quenching (NPQ). Coenzyme M (CoM) and ascorbate (Asc) displayed strong rapid effects—within 30 min—which were consistent with the direct role of Asc on violaxanthin de-epoxidase (VDE) activity: Asc servs as a cofactor that enhances VDE-mediated energy dissipation, observed in plant pigment changes. Interestingly, CoM appears to inhibit the same process. This appears to be the major mechanism for NPQ impacts, as protein levels of VDE, zeaxanthin epoxidase (ZEP), and PsbS remained unchanged, and neither treatment increased lumen acidification. Time resolved studies on the all six antioxidants also indicated slower effects, potentially through effects on endogenous antioxidant pools. Collectively, these results demonstrate that antioxidant application affects photosynthesis through multiple, compound-specific mechanisms and establish a mechanistic framework for interpreting antioxidant effects on photosynthetic resilience.

**Xin Wang**, FL-AES discussed recent findings in the group that the amino acid residue substitutions in the PSII reaction center protein D1 enhances photosynthesis and salt tolerance in cyanobacteria. These residue substitutions are located at the C-terminal tail of the precursor D1 (pD1), suggesting the pD1 processing step might be involved in improving photosynthesis in the mutants. To validate this, *in vitro* pD1 cleavage assay shows that one of the pD1 variants has significantly higher cleavage rate by its protease CtpA, suggesting that pD1 processing might be a limiting step under salt stress. Some preliminary studies by transferring the pD1-L353F variant into Arabidopsis support that it may also improve plant growth and salt tolerance. The group is currently working on validating the identified pD1 variants in conferring photosynthesis improvement and salt stress tolerance phenotypes.

**Ru Zhang**, MO-AES reported the investigation of heat induced cellular structural changes in the Green Alga *Chlamydomonas reinhardtii* by using cryo-volume electron microscopy (cryo-vEM). Cryo-vEM can preserve cellular structures under native conditions without any chemical fixation. It also allows 3D visualization of the spatial organization of cellular structures with 5 nm resolution. Algal cells with 8 h of heat at 35$^{o}$C, 40$^{o}$C or without heat treatments were cryogenically frozen in liquid nitrogen, milled and imaged using the Cryo Helios 5 Hydra cryo plasma-focused ion beam (FIB) scanning electron microscope (SEM) equipped with 3D vEM. With cryo-vEM, cellular structures can be viewed and quantified with different treatments. Thin lamellae from cryo-vEM can also be used for cryo-electron tomography to reveal heat effects on thylakoid membranes and the abundance/distribution of photosynthetic complexes at 1nm resolution. The information revealed with unprecedented precision can largely address knowledge gaps in the interactions among photosynthetic electron transports and thylakoid structures under stressful conditions, and help improve stress tolerance in photosynthetic cells.

**Nan Jiang**, HI-AES reported recent progress on elucidating the regulatory role of the MYB transcription factor MYB75/PAP1 (PRODUCTION OF ANTHOCYANIN PIGMENT 1) in *Arabidopsis*. MYB75 is known for regulating anthocyanin biosynthesis, but emerging evidence suggests that it may also play a role in chloroplast biogenesis, a key process in photosynthesis. The Jiang lab successfully reproduced the *myb75* mutant phenotypes previously observed, confirming a foundational step toward dissecting MYB75's dual function. Transcriptomic analysis, including RNA-Seq and GO enrichment, revealed that MYB75 regulates a subset of genes involved in chloroplast development, supporting its proposed role beyond pigment biosynthesis. We performed EMS mutagenesis in a *tt5* mutant background (deficient in anthocyanin production) and identified approximately 150 suppressor lines that partially restored chloroplast biogenesis. These lines exhibited a range of phenotypes from light to dark greenish seedlings, indicating genetic interactions influencing plastid development. The Jiang lab continues to characterize these suppressors to uncover new components linking anthocyanin pathway and chloroplast formation.

**Anthony Studer**, IL-AES gave a summary of his research interests and expertise as an incoming member of the group. The emphasis of his report was on progress related to the manipulation of leaf area in maize and the effect of canopy architecture on light interception and yield. The work included genetic mapping and the elucidation of the causative mutation in the *reduced leaf area* (*rdla*). The identification of a large-effect gene controlling leaf area in maize will permit further manipulation of the gene. Although progress has been made at the molecular level, alteration of leaf area was also evaluated in commercially relevant hybrid germplasm in multi-location trials with multiple planting densities and row spacing combinations to investigate the interaction between canopy architecture and management strategies. Ground truth measurements will be combined with drone images and yield data to build crop growth models that predict the performance of various genetic alterations of leaf traits under different planting densities and row spacing.

## Business Meeting

We discussed the future locations to led by Asaph Cousins with support from all others. For the next meeting, it will be held in the middle to late October time frame at Michigan State the following year, and at the next meeting we will discuss the renewal writing team and future proposal submission. Next year there will need to be an effort towards writing the renewal as the current grant ends 9/30/27. At the meeting it was discussed to try to continue to seek out new productive members who could participate in future meetings.

## Important upcoming dates

Letter to request a renewal due in September of 2026

Objective needs to be updated 2026

Due dates for renewal drafting
9/15/2026: Request to write a proposal
10/15/2026: Project Objectives and "Issues and Justification" due in NIMSS
12/1/2026: Full proposal due

Instructions for submitting the renewal can be found here.

1. **Request to Write a Proposal**: For NC renewals, start with requesting to write the renewal proposal. This is due by 9/15 the year before expiration.
- Contact the agInnovation North Central region multistate portfolio manager (chamilton@wisc.edu) and let us know your project wishes to renew, then we will set up the proper proposal template in NIMSS and give you a “temp” number that will stay with the proposal until it’s approved.
- We’ll also need to know who your AA will be and the names of anyone needing upload access to get started.

## Accomplishments

## Our objectives

Objective 1. Identify Strategies to optimize the assembly and function of the photosynthetic membrane.
Objective 2. Identify strategies to modify biochemical and regulatory factors that impact the photosynthetic capture and photorespiratory release of $CO_2$.
Objective 3. Identify strategies to manipulate photosynthate partitioning.
Objective 4: Develop strategies to overcome limitations to photosynthetic and plant productivity caused by management, developmental, and environmental factors

## Milestones & Activities:

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

- The Roston lab (NE-AES) identified multiple proteins as potential contributors to thylakoid membrane homeostasis. More than 50 are implicated by homology to have roles in lipid transport, one in membrane organization. Three candidate residents of membrane contact sites between the two membranes have been partially characterized and have altered association of the inner envelope with the thylakoid membrane.
- The Kirchhoff laboratory (WA-AES) has finalized its research on state transition in Arabidopsis. The manuscript is presently undergoing review. Our findings indicate that hyper-phosphorylation of a particular subset of light-harvesting complexes effectively equilibrates the electron transport rates of photosystem I and II, commencing from a highly imbalanced initial state prior to the transition. The synchronization of electron transport rates is imperative for ensuring both the efficiency and safety of photosynthetic energy conversion. Overexcitation of photosystem II may result in photooxidative damage mediated by reactive oxygen species, while overexcitation of photosystem I can lead to inefficient utilization of excess photons, thereby diminishing the efficiency of electron transport. This issue is especially pronounced under conditions of limited light.
- Another project in the Kirchhoff laboratory examined the influence of illumination on electron transport within thylakoid membranes in intact Arabidopsis plants. The study revealed that the linear electron transport rate increases by approximately 70% at light intensities exceeding roughly 100 µmol quanta per square meter per second. This enhancement develops with a half-time of approximately four minutes and relaxes at a considerably slower pace. Based on comprehensive characterizations of this phenomenon, it is hypothesized that the light-induced acceleration of electron transport results from large-scale protein reorganization within stacked membrane domains. It is further postulated that this reorganization facilitates the diffusion of plastoquinone from photosystem II to the rate-limiting cytochrome b6f complex. Consequently, this results in the additional involvement of cytochrome b6f complexes situated in remote, unstacked domains, namely, an increased count of rate-limiting enzymes that participate in linear electron transport, thereby explaining the observed increase in rates.

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

- The Cousins lab (WA-AES) has used leaf carbon isotope composition (δ13C) to determine how water use efficiency differs in two C4 species of Setaria species and in Sorghum mapping populations. This research helped to better understand the relationship of leaf carbon isotopes and the influence intrinsic water use efficiency to whole plant water use efficiency.

- The group also used $\delta^{13}C$ and maize genetic diversity to explore biochemical and post-photosynthetic factors that may influence $\delta^{13}C$. They found that the observed variation in $\delta^{13}C$ across diverse maize lines is likely driven by differences in CO2 availability and not photosynthetic or respiratory metabolism.
- Little is known about intraspecific variation mesophyll conductance ($g_m$), which describes the movement of CO2 from the intercellular air spaces to the site of initial carboxylation in the mesophyll, about in C4 plants. To address these questions, $g_m$ was measured by the Cousins Lab on numerous C4 species in response to CO2, employing three different estimates of $g_m$. Our results provide strong support for a CO2 response of $g_m$ in *Zea mays* and indicate that gm in maize is likely driven by anatomical constraints rather than biochemical limitations. The CO2 response of gm indicates a potential role for CO2-transporting aquaporins in C4- $g_m$. These results also suggest that water-use efficiency could be enhanced in C4 species such as maize by targeting $g_m$.
- If $g_m$ were to limit C4 photosynthesis, it would likely be at low CO2 concentrations (pCO2); however, data on C4- $g_m$ across pCO2 are scarce. The Cousins lab has described the response of C4- $g_m$ to short-term variation in pCO2, at three temperatures in Setaria viridis, and at 25 ºC in Zea mays. Additionally, the lab has quantified across pCO2 the potential limitations to photosynthesis imposed by stomata, mesophyll and carbonic anhydrase (CA) and the effect of finite $g_m$ calculations of leakiness. In both species, $g_m$ increased with decreasing pCO2. At pCO2 below ambient, photosynthetic rate was limited by CO2 availability. In this case, the limitation imposed by mesophyll was similar or slightly lower than stomata limitation. At very low pCO2, CA further constrained photosynthesis. High $g_m$ could increase CO2 assimilation at low pCO2 and improve photosynthetic efficiency under situations when CO2 is limited, such as drought. Finite $g_m$ increased estimates of leakiness over values derived with $g_m$ infinite in Setaria but not in Zea.
- The Cushman lab (NV-AES) continued its efforts to engineer synthetic crassulacean acid metabolism (SynCAM) in Arabidopsis and soybean (*Glycine max*). Arabidopsis plants expressing the carboxylation module and core CAM modules consisting of seven or nine genes showed significant increases in rosette diameter and leaf fresh weight, leaf thickness, and leaf succulence as well as significant increases in dawn/dusk titratable acidity, malate content, soluble carbohydrates, and starch accumulation. In contrast, plants expressing the decarboxylation module showed no improvement in plant biomass and no increase in leaf thickness, leaf succulence, dawn/dusk titratable acidity, malate content, soluble carbohydrates, and starch accumulation. Acute water-deficit (drought) tests of Arabidopsis plants expressing the decarboxylation module and core CAM modules consisting of seven or nine genes showed significant improvements in survival rates, whereas no such improvement occurred in plants expressing the carboxylation module alone. Interestingly, plants expressing the carboxylation module and core CAM modules consisting of seven or nine genes also showed significant improvement in heat stress tolerance. Similar results of improved biomass production and water-deficit stress tolerance are also observed in soybean.

**Objective 3. Identify strategies to manipulate photosynthate partitioning.**

- The Giroux Lab (MT-AES) investigated the relationship between wheat productivity, photosynthetic performance, and starch metabolism. The team evaluated recombinant inbred line (RIL) populations that varied in leaf starch content over two field seasons. Leaf starch content was found to correlate negatively with flowering time, plant biomass, and seed yield. This RIL population was subsequently used for quantitative trait loci (QTL) mapping, which identified a locus on chromosome 3D associated with leaf starch, flowering time, and yield. Fine-mapping and candidate-gene analyses are being conducted to identify the gene underlying this QTL. Once identified, gene-specific markers will be developed to support selection for improved plant productivity. The overall goal of the project is to advance breeding strategies that enhance photosynthesis, starch utilization, and crop performance.
- The Melis Lab (CA-AES) continued to address the problem of heterologous protein over-expression in photosynthetic systems. They worked with *Synechocystis* sp. PCC 6803 (*Synechocystis*), a unicellular photosynthetic microorganism that is a model for photo-biochemical research. A severe limitation in synthetic biology objectives is the low cell tolerance of heterologous proteins, which are readily degraded by the cellular proteasome. The Melis lab showed that fusion constructs of recombinant proteins with the light-harvesting CpcA α-subunit or CpcB β-subunit of phycocyanin in *Synechocystis*assemble as modified $(\alpha,\beta^*P)_3$ heterohexameric discs, where both the phycocyanin $(\alpha,\beta)_3$ trimer and the fused heterologous enzyme *I* protein P stably accumulate, while retaining their respective native catalytic activities. In this period, the work focused on expressing in *Synechocystis* the Fibroblast Growth Factor 2 (FGF2), an important signaling protein that serves to activate a variety of biological processes in animal and human cells. The FGF2 comprises an uncommon β-barrel structure composed of β-pleated sheets and loops only. It functions as a multipurpose protein, involved in cell growth, division, and repair. Accordingly, FGF2 is important in the biopharmaceutical / biomedical, as well as applied research fields. However, stable FGF2 expression and isolation in heterologous systems is hindered by the exclusively β-sheets structure of this protein. To stabilize and enhance accumulation of the FGF2 in *Synechocystis*, the codon optimized *FGF2* gene was installed as a fusion construct with the highly expressed CpcB β-subunit of phycocyanin. This enabled stable recombinant phycocyanin-FGF2 fusion protein accumulation in *Synechocystis*, overcoming the difficulty due to the occurrence of β-sheets only in the structure of this protein. Furthermore, the recombinant phycocyanin-FGF2 fusion protein (Phyco*FGF2) exhibited both phycocyanin and FGF2 bioactivity. The work supports the notion that cyanobacterial cells will tolerate difficult to express heterologous recombinant proteins, when fused to phycocyanin, as the latter is needed for photosynthesis and cellular growth, thus enabling a stoichiometric accumulation, in this case between phycocyanin and the FGF2 protein.

**Objective 4: Develop strategies to overcome limitations to photosynthetic productivity caused by developmental and environmental factors**

- The Studer lab (IL-AES) assessed wildtype and narrow leaf hybrids (created using the *rdla* mutation) during the 2025 field season. Hybrids were planted at 74k, 89k, and 104k

plants per hectare in 76 cm row spacing, and 104k, 119k, and 133k plants per hectare in 51cm rows.

- The Studer lab (IL-AES) discovered the causative mutation underlying the *reduced leaf area* phenotype, which will enable further canopy architecture manipulation.
- The Studer lab (IL-AES) characterized the effect of the *rdla* mutation on each leaf throughout development in multiple genetic backgrounds. These results give insight into the genetic factors controlling leaf area from the bottom to the top of the canopy that gives rise to unique canopy shapes.
- The Harper lab (NV-AES) provided evidence that changes in the basal levels of cytosolic $Ca^{2+}$ can activate a programmed cell death pathway in plants. Insights into how basal levels are controlled are expected to guide future efforts to engineer plants to be more productive under temperature-stress conditions.
- The Harper lab (NV-AES) continues to find evidence for differences in how pollen and vegetative cells sense and respond to heat stress. This is significant because it 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).
- The Cushman lab (NV-AES) generated soybean (*Glycine max*) lines with improved water-deficit (drought) tolerance through tissue succulence engineering using a basic helix-loop-helix transcription factor from winegrape under the control of a strong constitutive promoter. The engineered lines showed significant improvements in acute water-deficit (drought) stress. Physiological studies revealed that the drought tolerance arose from a combination of increased water availability within leaves and increased water-use efficiency due to reductions in stomatal density and conductance. Tissue succulence engineering also increased seed size and yield and seed protein and oil content when plants were grown under unstressed conditions.
- The Glowacka lab (NE-AES) NPQ, by stimulating thermal dissipation of excitation energy, can modulate redox state of chloroplastic quinone A (QA), a primary electron acceptor of photosystem II (PSII), which is an early signal for light-induced stomatal opening. Soybean lines with upregulated NPQ exhibited more oxidized QA across the range of light intensities. Overexpression of PsbS in soybean under one of three promoters selected to be responsive to different environmental cues such as light intensity, drought or metabolomic feedback from photosynthesis led to increase in NPQ under high light intensities. Higher NPQ translated to more oxidized redox state of QA in all three transgenics. This relation between NPQ and QA redox state was confirmed in preliminary field test under rainfed conditions in East Nebraska and led to lower stomatal conductance in line in which promoter selected to be responsive to drought was used. Seed yield is to be esteemed by processing harvested from field plants. Soybean stands out as a species with relatively narrow variation in NPQ kinetics therefore (1) soybean might benefit from NPQ engineering to increase abiotic stress adaptation and that (2) NPQ improvements are likely to be best achieved through transgenic approaches.
- The Wang Lab (FL-AES)employed directed evolution in cyanobacteria to isolate beneficial genetic traits that improve photosynthesis under stress conditions. We found that amino

acid substitutions in the PSII reaction center protein D1, specifically in the precursor D1 (pD1) tail, have a significant impact on photosynthetic efficiency under salt stress. We identified three residue substitutions in the pD1 tail, L353F, I358N, and H359N, that produce this effect. Preliminary evidence from transgenic *Arabidopsis* plants overexpressing the cyanobacterial pD1 L353F variant suggests that it also enhances plant growth and salt tolerance.

## Outputs:

See list of Publications below.

## Plans for the Coming Year:

**Objective 1:**

- The Roston lab (NE-AES) is refining impact of two membrane contact site candidates between the chloroplast inner and thylakoid membranes. We will test their ability to transport lipids and their sub-organellar location. Further, we are preparing a manuscript to publish the screens through which we identified these candidates.
- The Kirchhoff laboratory (WA-AES) will further characterize the mechanism underlying the light-triggered acceleration of linear electron transport rates in Arabidopsis. Additionally, we will conclude electron microscopic examinations of light-induced modifications to the overall thylakoid architecture. Finally, we are in the process of publishing a project concerning the visualization and mathematical analysis of the supramolecular protein arrangement within stacked grana thylakoid domains.

**Objective 2:**

- The Cushman lab (NV-AES) will continue its efforts to engineer synthetic crassulacean acid metabolism (SynCAM) in Arabidopsis and soybean (*Glycine max*). These studies will continue to investigate the physiological basis for improved photosynthetic carbon capture and resultant plant productivity through the addition of SynCAM modules and test how these modules improve plant productivity and alter plant anatomy under normal and drought and heat stress conditions.

**Objective 3: Mechanisms regulating photosynthate partitioning**

- The Melis Lab (CA-AES) will apply the ***phycocyanin fusion constructs*** technology to further investigate the basic aspects of recombinant protein stability and over-expression observed. The objective would be to optimize the process by better defining the parameters that result in over-expression. The research will investigate the role of protein folding patterns in the post-translational stability and the distancing of phycocyanin from the exogenous fusion protein via the use of different length and conformation oligopeptide spacers.

- In collaboration with a major Biotech Ag company, maize lines expressing variants Pho1ΔL80 have been generated. The study of these maize lines will shed light on whether there are differences in Pho1 function in a C4 environment.
- The Roston lab, in collaboration with the Glowacka and Buan labs, is testing antioxidant application and its effect on carbon partitioning of photosynthate.

**Objective 4:**

- The Harper lab (NV-AES) will continue to investigate how changes in basal cytosolic $Ca^{2+}$ concentrations change a plant's response to the environment.
- The Cushman lab (NV-AES) will continue work on phenotyping soybean plants engineered for increased tissue succulence and water-deficit (drought) and heat stress tolerance.
- The Cushman lab (NV-AES) will continue work on phenotyping Arabidopsis and soybean plants engineered for decarboxylation, carboxylation, and core SynCAM modules for traits associated with improved biomass production and water-deficit (drought) and heat stress tolerance.
- The Giroux lab (MT-AES) will conduct functional testing of genes associated with leaf starch variation that is linked to plant productivity.
- The Walker lab will continue characterizing photorespiratory genes from species adapted to high and low temperatures.
- The Glowacka lab (NE-AES) will explore the effect of different promoters to drive a transgenic allele designed to express the photosystem II subunit S, as a means to modulate chloroplast-derived signal for stomata opening. Define NPQ-H2O2 relations for effective coordination between stomatal conductance and photosynthesis.
- The Wang lab (HI-AES) will dissect the detailed molecular mechanism underlying improved photosynthesis in the pD1 variant strains in cyanobacteria. The outcome will guide future efforts in engineering these genetic traits into crops to improve photosynthesis and growth.

## Impacts

**Objective 1:**

- Rebecca Roston (NE-AES) published a review that clarifies the sub-cellular compartmentation of plant lipids. **Also, the progress on targeting lipid transport to the thylakoid membranes resulted is supported from the Department of Energy, BES "Photosynthetic membrane lipid transport through chloroplast membrane contact site homologs", 2023 – 2026.**
- The overarching goal of the Kirchhoff lab (WA-AES) is developing a mechanistic understanding of structure-function relationships in plant photosynthetic membranes. The lab covers length scale from sub-nanometer to micrometer. Knowledge from this research is a key element for the identification of strategies to improve crop resilience in changing environmental settings. **Research projects in the Kirchhoff lab are supported by**

**grants from the Department of Energy (BES), The National Science Foundation (MCB), and USDA-NIFA.**

**Objective 2:**

- The Cousins lab (WA-AES) receives funding for their work from the USDA, DOE BER, DOE BES, and the Gates Foundations. **The collaborations and engagement in the NC1200 has provided support and complements our federally funded research.**

**Objective 3:**

- A general guiding principle in the field of biology posits that heterologous gene overexpression in photosynthetic systems is satisfied solely upon the selection of a strong promoter under the control of which to express the desired recombinant protein. In the vast majority of such eukaryotic gene overexpression efforts in the literature, however, the corresponding target protein cannot be detected in Coomassie-stained SDS-PAGE and its presence, in trace steady-state amounts, is evidenced with indirect methods only, such as sensitive Western blot analysis, suggesting that eukaryotic gene expression under the control of a strong promoter does not in fact translate into substantial amounts of the target protein in photosynthetic systems. This barrier in the overexpression of heterologous eukaryotic proteins in photosynthetic tissues is evidenced widely in the literature. **The Melis Lab (CA-AES)** contributed with the design of oligonucleotide fusion constructs, as functional protein overexpression vectors in photosynthetic cyanobacteria to overcome this pitfall. The fusion constructs technology was successfully applied in the overexpression of plant terpene synthases, the human interferon, fibroblast growth factor 2, and the bacterial tetanus toxin fragment C in cyanobacteria. True overexpression of these plant, human, and bacterial origin genes to levels up to 10-20% of the total cellular protein were demonstrated. A patent and two papers were published in this period (please see below).
- Rebecca Roston (NE-AES), Nicole Buan (NE-AES) and Kasia Glowacka (NE-AES), and have published two patents describing the application of Coenzyme M to plants, and the production of it in bacteria. **2020-077 Application number, 63/659,175. Use of a small, effective antioxidant to increase plant and microbial biomass. 2024-006 Application number 63/659,271 Synthetic operon for the production of 2-mercaptoethane sulfonate (coenzyme M).**
- Mike Giroux (MT-AES) identified a major QTL in wheat linked to leaf starch, flowering time, and yield demonstrating how natural variation, particularly the FT-D1 gene located within the major QTL on chromosome 3D, can significantly influence wheat performance under changing environmental conditions**.**

**Objective 4:**

- The Harper lab (NV-AES) created a genetically encoded ratio-metric ATP sensor by fusing a MaLion-Red ATP sensor to a Neon-Green normalization reference. The ratio-metric feature allows the status of cellular ATP concentrations to be compared between different

cell types. This reporter is being used to evaluate the connection between basal levels of calcium and the regulation of energy homeostasis under a variety of environmental situations, including heat stress.

- Research efforts by the Cushman lab (NV-AES) for tissue succulence engineering represent an innovative strategy for improving biomass and reproductive yields under normal conditions in both Arabidopsis and soybean (G*lycine max)*, water-deficit (drought) stress tolerance, and water-use efficiency with potential applications to other crops.
- The Cushman lab (NV-AES) made significant progress towards engineering SynCAM in the model species in both Arabidopsis and soybean (G*lycine max)* in collaboration with the Wisconsin Crop Improvement Center. Installation of carboxylation module and core CAM modules was used to increase biomass production, whereas the installation of a decarboxylation module and core CAM modules were used to improve water-use efficiency and drought tolerance with potential applications to other crops.
- The Zhang lab (MO-AES) studies how photosynthetic cells respond to high temperatures by using both green algae and land plants as models. Our ultimate goal is to engineer more efficient and robust photosynthesis under high temperatures for improved agricultural production and biomass accumulation. We 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. In collaboration with the Morgan-Kiss lab, we also investigated CEF in both psychrophilic and mesophilic Chlamydomonas species. We are currently investigating heat effects on thylakoid structures in Chlamydomonas reinhardtii by using multiscale Cryo-Volume Electron Microscopy and Cellular Cryo-Electron Tomography. Additionally, we generated transgenic mutants with altered photoprotection (non-photochemical quenching, NPQ) in the $C_4$ model plant *Setaria viridis* and investigated the regulation of NPQ in $C_4$ model plants. These results help us understand the regulation of $C_4$ photosynthesis and provide insights for improving photosynthesis in $C_4$ crops.
- The Glowacka lab (NE-AES) studies resistance of photosynthesis to abiotic stresses. Our ultimate goal is to engineer more efficient and robust crops for food, feed and biomass. Studies on resistance of photosynthesis outlined here and performed in the Glowacka lab NE-ARS, were supported through **NSF CAREER grant "CAREER: Understanding non-photochemical quenching under chilling in the warm-season C4 grasses", Award OIA-2142993, (2022-2027), $1,375,334**. The progress on engineering the better water use efficiency was supported by **Nebraska Soybean Board, "More soybean for less water: genetic approach for improving water use efficiency", (2024-2025), $46,407**.
- Xin Wang (MT-AES) has ongoing collaboration with Ru Zhang working on a DOE-funded project to dissect the role of PSI supercomplexes under stress in the psychrophilic algae *Chlamydomonas prescuii*. Xin Wang joined the NC1200 group in 2024 and work in his group on pD1 processing in cyanobacteria will likely lead to new findings to help create robust photosynthesis in cyanobacteria and plants. The long-term goal is to translate the knowledge found in cyanobacteria into crop plants to increase photosynthesis and crop yield. **This work is partly supported by an NSF-IOS CAREER grant. X Wang "Glycogen metabolism kick-starts photosynthesis in cyanobacteria" 2021-2026.**

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