Basic Information

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Participants

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Brief Summary of Minutes of Annual Meeting

The meeting was held at the Donald Danforth Plant Science Center in Saint Louis, MO. Doug Allen (USDA-ARS) began the meeting with introductions.

Rob Aiken, KAES presented on a series of whole-plant to field studies: 13C and genetic selection in wheat, UAV remote sensing platform and genetic variability in wheat, a simple model for biomass accumulation based on canopy energy and water balance, field measure of gas exchange in a C4 (biomass sorghum) and C3 (perennial Silphium oilseed) and a collaborative effort with the Melis lab on screening for Truncated Light-harvesting Apparatus (TLA) in sorghum.

Doug Allen, MO-ARS with the USDA lab at the Danforth Center reported on ongoing investigations in C3 and C4 leaf metabolism and mentioned other studies in seed, algal and cyanobacterial systems – all related to photosynthetic carbon assimilation and partitioning. The lab is studying tobacco leaves with dramatically increased lipid production which impact carbon partitioning to starch and may recapitulate a carbon concentrating mechanism in seeds due to the heightened CO2 production from making lipids. In C4 studies, PEPCK has been knocked down in maize lines to investigate the role of minor decarboxylation pathways on crop productivity under altered environmental conditions.

Christoph Benning, MI-ABR described the PLIP (Plastid Lipase) role in membrane lipid composition changes with PG 18:3 cleavage that gets exported and placed on PC. So, a fad3 in the ER is rescued by plastid targeted lipase. PLIP2 and 3 target other lipids. The PLIPs are relevant for abiotic stress as indicated by ABA provision tests. ABA induces JA production in WT, but not in PLIP mutants. The PLIPs connect abiotic and biotic stress by clipping PUFAs converted to OPDA that is exported and converted to JA. Work is significant because abiotic and biotic stresses often contribute susceptibility to each other.

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.

Felix Fritschi, MO-AES is investigating the natural variation in light capture and photosynthetic light reactions that is present in diverse maturity group IV soybean genotypes. Previously, they have characterized the leaf pigments using wet chemistry and canopy reflectance measurements and successfully identified genomic regions associated with leaf and canopy pigmentation of soybean. Current efforts have been focused on chlorophyll fluorescence phenotypes which were assessed in a diversity panel of nearly 200 soybean genotypes. Genome wide association analysis was conducted to identify genomic regions associated with 21 chlorophyll fluorescence genotypes. These analyses identified a total of 53 putative loci, 13 of which were associated with three or more chlorophyll fluorescence phenotypes.

Glenda Gillaspy, VA-AES reported on role of inositol pyrophosphates on sugar signaling in plants and impact on photosynthesis. Inositol phosphates and pyrophosphates link to the energy status of the cell amongst other things. Bifurcation of pyrophosphate production results in 1- and 5-InsP7 intermediates. VIP1 produces the 1-InsP7 and contains a kinase and phosphatase domain. Glenda described the role of VIP1 and 2 that changed the InsP7 to InsP8 ratio, with VIP being primarily to convert 7 to 8 not 6 to 7. In addition, she described phosphate limitation that is a future problem with solutions posed by reducing IPK1 or modulating the VIP genes that alter phosphate starvation or replete sensing. InsP7 presence or absence enables/represses starvation response.

Jeff Harper, NV-AES described flippases, amino lipid stimulated P type ATPases (ALA) for lipids. KO of lipases ALA4/5 are important for catabolism of sphingolipids. Some of the ALA 6/7 affect pollen formation, flipping lipids are important for plant biomass. Traditionally, lipid flippases are thought to be involved in creation of lipid asymmetry between membrane leaflets. ALA4/5 may be involved in flipping glucosylceramides inside the plasma membrane, which could then be subject to the action of cerimidases. This could alternatively occur on the ER. Without ala4/5, glucosylceramides would accumulate in the plasma membrane and signal. ALA10 seems to be a phospholipid uptake system. In roots, it can move lipids into roots from the environment.

Jiaxu Li, MS-AES Silicon can improve growth and drought tolerance in soybeans. Two-dimensional gel analysis was performed to see what proteins were impacted as a consequence of silicate application, also with a drought condition. Proteins that showed up under drought condition may be important for abiotic stress tolerance (pathogenesis related protein). Several hypothetical peroxidases showed up in the non-drought condition. The effects of silicate application on plant dry weight of two soybean varieties results in increased yields compared to application of potassium chloride fertilizer. These results were replicated in the greenhouse, outside and in full field conditions.

Tasios Melis, CA-AES investigated reducing antennae size in either tobacco or Chlamydomonas which results in an increase in total photosynthetic capture, because of the increase in light penetrance. This was mediated by knocking down the thylakoidal SRP pathway. Genetic determinants that establish chlorophyll antenna assembly and size, were assessed by a mutant screen. Developed approaches to limit antenna size since 80% of harvested light in Chlamy at the surface of 'donut' reactor was lost by NPQ and only ~20% was effectively used for photosynthesis. Identified TLA gene mutants, investigated in tobacco, resulted in reduced coloration, alterations in antenna size (reduced by 50%), ratio of Chla/b changed from 3:1 to 8:1 as a result of no periphery antenna formation. TLA3-RNAi lines indicated light saturation at increased light intensities per unit area. However, investigation of oxygen evolved for changes in light intensity indicated near identical initial slopes indicating the reduced antenna size did not compromise photosynthesis. Inspection of the internode distances in the TLA-RNAi indicated increased biomass per unit area. Ratios of total biomass to WT, Leaf biomass to WT, Stem biomass relative to WT all went up and Leaf to Stem biomass increased significantly too. Estimates that planting density can be tripled and may also minimize losses of water loss, fertilizer lost, and weed formation.

Rebecca Roston, NE-AES made measurements in three related grass species during cold showed strong dyal cycles of the three major membrane lipids phosphatidylcholine, monogalactolipid and digalactolipid. The extent of the cycling is larger than the change at the same time of day. Sensing of cold in warm-adapted species seems to occur at warmer temperatures, but the effects are attenuated. Finally, the chloroplast is likely to have membrane contact sites with other organelles and within itself, and it has homologs of many membrane-contact site lipid-transfer proteins.

Tom Sharkey, MI-ABR reported on chloroplast glucose-6-P metabolism inside the chloroplast. How is carbon exported from the chloroplast? Daytime, triose phosphate export using the triose phosphate antiporter TPT. The logic is that at night, when ATP is expensive, exporting G6P would save ATP because it would save a re-phosphorylation of maltose or glucose. However, direct transport is unlikely because there is more G6P in the cytosol than in the stroma. This is true at most times. Thus, GPT2 (G6P transporter) brings G6P into the stroma rather than out. When starch synthesis is limited (phosphoglucomutase KO or GWD KO, both of which cannot access starch stores), GPT2 transcript levels are increased. GPT2 expression increases 1000X with an increase of light intensity from 500 to 1000 umol/m2sec2. G6P levels in the chloroplast must remain low to limit the amount of the G6P shunt that occurs under normal conditions. GPT2, the transporter for G6P is critical in a variety of mutant backgrounds. The shunt may be important during the evening and the mutants may be activating a signal from the chloroplast that is asking for import of G6P.

In addition, we had several guests present brief talks of about their scientific efforts related to photosynthesis:

Katarzyna Glowacka reported that modification of the NPQ component can improve the dynamic recovery from photoprotection. In fact, increasing recovery from photoprotection could result in 15% improved photosynthesis. This is indicated by recovery of CO2 assimilation measurements. The improvement is relevant during fluctuating light conditions, due to faster relaxation and less NPQ. The results were confirmed in field studies with dry weight increases of 11-20%. NPQ can also improve water use efficiency. Overexpression of PSbS can lead to less stomatal opening in response to light and unchanged co2 assimilation which improved WUE by 25%.

Elizabeth Kellogg discussed the function of the pedicellate spikelet and awn in Sorghum and related grasses. Spikelets in grasses are in pairs. Only one spikelet in the pair makes a seed. What is the awn doing? Observations from sorghum: sterile spikelets are green, awns are brown (in sorghum), but there's evidence that it is photosynthetic in wheat. Could the rationale be photosynthetic? 14C labeling - Do spikelets take up any carbon? She and the Allen lab exposed inflorescences (whole or dissected) to 14C. After 1 hour, the Awn had taken up less than .5% of counts, the sterile spikelets, 89% of counts and seed producing 9.8%. After 24 hours, the awn increased to 3%, the sterile spikelet went down and the seed producing spikelet increased. Similar results were produced in other species. There is leaf-like morphology to the sterile spikelet including lots of stomata, this is missing except for margins from the seed-producing spikelet and the awn. Metabolite and transcript analysis shows that the awn and the sterile spikelet are much more similar than the sterile spikelet.

Ru Zhang reported on improved heat tolerance for algal and crop yields. How do photosynthetic cells sense and regulate heat responses? What genes are required for heat tolerance and are their differences between moderate and acute heat stress. She is using algal mutant collection of 62000 individual bar-coded gene knockouts to inspect performance in heat stress conditions and prioritize candidate heat genes from the list, analyze RNA-seq and do metabolomics.

Business meeting. We discussed the future location. Next year the plan is Washington, either Seattle or Spokane. In two years we are likely to have at Michigan State. The attendees voted to accept Katarzyna Glowacka as a new member. Ru Zhang is interested in joining as well through her U of Missouri faculty appointment and will submit a CV to be further circulated.

Accomplishments

Milestones & Activities:

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

1. The Benning lab has completed and published the analysis of two paralogs of PLIP1 in Arabidopsis, PLIP2 and 3. These are also chloroplast lipases targeting specific thylakoid membrane lipids. We were able to show their activity in vivo and in vitro. The expression of the two respective genes is induced by the plant abiotic stress hormone ABA. Overexpression of the respective cDNAs in Arabidopsis caused severe stunting of the plants especially in case of PLIP2. As a cause, we determined that these plants accumulate oxylipins, which leads to the induction of biotic defense pathways. Introducing a mutation that causes the loss of the JA-Ile receptor COI1 in Arabidopsis reversed the growth defect in the PLIP cDNA overexpression lines. We also constructed triple mutants lacking the three isoforms PLIP1, 2, and 3. The seeds and seedlings showed an increased sensitivity to ABA. Based on our findings on PLIP2 and 3, the lab proposed a new model that places these two plastid lipases at a key junction between abiotic and biotic stress responses.
2. To utilize the PLIP1 pathway for the engineering of seed oil content in a potential crop plant, the Benning lab expressed the PLIP1 cDNA under the control of a seed-specific promoter in Camelina. A large number of transgenic plants was obtained that we are now studying in the T3 generation. The growth of these plants seems normal and preliminary results suggest that oil content per seed may be slightly increased, although seed number per plant may be slightly reduced. More importantly, overexpression of the PLIP1 cDNA caused a change in seed oil fatty acid content towards fatty acids with shorter chain length and fewer double bonds. PLIP1 cDNA overexpression in a seed-specific manner led to Camelina oil similar to that of Canola.
3. The Benning lab advanced the analysis of the reaction mechanism of the unusual FAD4 desaturase of Arabidopsis and are currently preparing a publication on this topic. 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, which is the substrate for PLIP1 mentioned above. Loss of this lipid in the fad4 mutant reduces seed oil content in Arabidopsis. 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 were identified that are essential for its activity. Based on these results, the lab is proposing a hypothesis linking the redox state of the chloroplast to the activity of the FAD4 desaturase.

4. The Benning lab is also studying a novel rhomboid protease of Arabidopsis located in the inner chloroplast envelope membrane. The biochemical analysis of the mutant indicates that the supply of lipid precursors from the inside of the chloroplast to the enzyme that assembles the galactolipid monogalactosyldiacylglycerol is disrupted, while the precursor supply from the ER compensates. Label experiments with isolated chloroplasts show a buildup of phosphatidic acid and a decrease of label in the galactolipid. However, neither phosphatidic acid phosphatase in the chloroplast nor the galactolipid synthase are decreased in their activity in the mutant. Based on these results the lab proposes a new hypothesis that takes into account the topology of the enzymes and we postulate that phosphatidic acid transfer from the inside of the inner chloroplast envelope membrane to its intermembrane face is disrupted. How the rhomboid protease affects this process is currently being investigated and one clue will come from the analysis of proteins associated with it in a large complex of the inner chloroplast envelope membrane.
5. The Benning lab published a study of transgenic Arabidopsis plants expressing the cDNA encoding an ER delta 6 desaturase to tag ER lipids and monitor their import in to plastids. Key findings from this study were that a fraction of phosphatidylglycerol in the chloroplast is derived from imported lipid precursors and that acyl editing on chloroplast lipids is extensive.
6. As per the multistate metrics, DNA plasmids expressing photosynthetically active genes are under construction in the Roston lab. Potential genes involved in thylakoid biogenesis have been cloned into vectors that fuse them with fluorescent proteins.
7. The lateral distribution of photosynthetic protein complexes between stacked (grana) thylakoid domains and unstacked areas controls essential functions, regulations, and maintenance of the energy transforming machinery in plants. The Kirchhoff lab established a toolkit for quantification of this lateral distribution between stacked and unstacked membrane domains. A special focus is on quantification structural dynamics of the photosystem II – light-harvesting II (PSII-LHCII) supercomplex that is in constant turnover due its high vulnerability for photo-oxidative damage (PSII repair cycle). Our quantitative analysis reveals that different thylakoid domains perform different functions for the PSII repair cycle with the stacked grana core harboring fully assembled and functional PSII, the margins of the grana is the place of disassembly and degradation of damaged PSII, and the unstacked stroma lamellae is the PSII repair zone.
8. The lateral distribution of central electron transport complex, cytochrome b6f (cyt b6f) complex, was studied by the Kirchhoff lab for light- and dark-adapted plants. It turns out that a certain fraction of this complexes moves from stacked to unstacked regions in a light intensity dependent manner. Furthermore, we find that this migration is dependent on protein phosphorylation since it is absent in thylakoid kinase knock-out mutants. This lateral reorganization of the cyt b6f complex could be key process to fine tune linear and cyclic electron transport processes to adjust ATP production in photosynthesis to the actual metabolic demand.
9. The role of thylakoid ion transporter/channels for controlling architectural alterations of the thylakoid network was studied by the Kirchhoff lab employing available knock out mutants for these transporter/channels and generation of all possible permutations of single mutants. The functional characterizations of these mutants demonstrate that they have specific functions in photo-protective high energy quenching (qE) and the partitioning of the electrical and chemical components of the proton motive force (pmf) across thylakoid membranes. A computer model to simulate ion fluxes in mutants and its implication on energy transformation is in progress.

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

1. The Fritschi lab characterized chlorophyll fluorescence phenotypes in a diversity panel, providing insights into the extent of natural variation present in soybean photosynthetic light reaction characteristics and identified genomic regions associated with 21 chlorophyll fluorescence phenotypes based on genome-wide association analysis.
2. The lab also continued examination of chlorophyll fluorescence of a chronosequence of soybean cultivars to determine the impact of breeding for high yield on photosynthetic light reactions.
3. In the Sharkey lab, a comprehensive study of the effect of a light jump on expression of the gene coding for the glucose-6-phosphate transporter (GPT2) was completed. RNA-seq was carried out and analyzed. Based on data from the RNA-seq we showed that the Redox Responsive Transcription Factor (RRTF1) was necessary for the GPT2 response to a light jump but it was not sufficient. Plants lacking the triose phosphate transporter (*tpt*) showed a strong response of RRTF1 but not GPT2 to a light jump.
4. The lab also measured the activity of glucose-6-phosphate dehydrogenase in crude extracts from leave was measured. While the activity was less in extracts from leaves in the light than from leaves in the dark, there was still significant activity indicating that when glucose 6-phosphate is present in the stroma some will be acted on by G6PDH initiating the glucose-6-phosphate shunt around the Calvin-Benson cycle.
5. Finally, the Sharkey group tested the release of carbon from glucose metabolism in the dark. They hypothesize that the respiration that can be measured (with difficulty) in the light in photosynthesizing leaves results, in large measure, from the G6P shunt. We fed glucose to leaves labeled in the C1 position or C2 and collected the CO_2 released to measure radioactivity. Very little label was released, and it was the same whether the glucose was

labeled in the C1 or C2 position. However, if fed glucose to the leaf for a day to induce GPT2 so that G6P could be taken up by chloroplasts we found more label released when C1 labeled glucose was fed than when C2-labeled glucose was fed.

6. The Cousins lab 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.
7. 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.
8. 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 five maize lines 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.
9. 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.

Objective 3. Identify strategies to manipulate photosynthate partitioning.

1. Evolution of sizable arrays of light-harvesting antennae in all photosynthetic systems confers a survival advantage for the organism in the wild, where sunlight is often the growth-limiting factor. In crop monocultures, however, this property is strongly counterproductive, when growth takes place under direct and excess sunlight. The large arrays of light-harvesting antennae in crop plants cause the surface of the canopies to over-absorb solar irradiance, far in excess of what is needed to saturate photosynthesis and forcing them to engage in wasteful dissipation of the excess energy. Evidence in work done by the Melis lab showed that downregulation by RNA-interference approaches of the Nicotiana tabacum signal recognition particle 43 (SRP43), a nuclear gene encoding a chloroplast-localized component of the photosynthetic light-harvesting assembly pathway, caused a decrease in the light-harvesting antenna size of the photosystems, a corresponding increase in the photosynthetic productivity of chlorophyll in the leaves, and improved tobacco plant canopy biomass accumulation under high-density cultivation conditions. Importantly, the resulting TLA transgenic plants had a substantially greater leaf-to-stem biomass ratio, compared to those of the wild type, grown under identical agronomic conditions. The results are discussed in terms of the potential benefit that could accrue to agriculture upon application of the TLA-technology to crop plants, entailing higher density planting with plants having a greater biomass and leaf-to-stem ratio, translating into greater crop yields per plant with canopies in a novel agronomic configuration.
2. Identification of a novel strategy that improves the photosynthetic output growth is under current pursuit in the Roston Lab.
3. Related to seed and leaf AGPase research, the Giroux lab completed creation of wheat transgenic events with increased leaf and seed AGPase and has planted greenhouse yield trial to measure how increased seed AGPase impacts wheat productivity under different soil fertility levels.
4. Regarding wheat reduced height research the Giroux lab has conducted field trials in which we measured plant growth throughout the season in NILs varying for the presence of the Rht-B1b mutation and measured photosynthesis under field conditions along with leaf and seed metabolites. The lab found that *Rht-B1b* mutations: i) reduce flag leaf photosynthesis by 18% and chlorophyll by 23%, ii) decrease individual seed weight beginning at 21 days post anthesis, and iii) do not largely impact whole plant carbon and nitrogen metabolism.
5. The Gillaspy lab has created DNA plasmids which can be used to express gene fusion constructs for production of recombinant proteins used to study inositol pyrophosphates in plants and has produced and characterized transgenic Arabidopsis plants with alterations in phosphate sensing. Data was produced on lipid metabolism and remodeling in mutants in response to varying phosphate levels. Data was produced on RNA regulation and signal transduction of key mutants in the inositol pyrophosphate pathway.
6. In addition, the Gillaspy lab coordinated an outreach activity to over 250 9th graders, who were engaged in STEM authentic inquiry using inositol pyrophosphate mutants and provided educational activities in a summer NIFA REEU program to expose diverse undergraduate students to translational plant science.

7. The Harper lab identified two lipid flippases, ALA4 and ALA5, that are critical for cellular expansion and vegetative growth in Arabidopsis thaliana. A double KO of ALA4/5 results in dwarfism, characterized by reduced growth in rosettes (6.5-fold), roots (4.3-fold), bolts (4.5-fold), and hypocotyls (2-fold). Multiple vegetative cell types showed reductions in cell size, suggesting a role for ALA4/5 in cellular expansion. Assays in yeast revealed that ALA5 can transport specific glycerolipids, as well as a sphingolipid. Lipid profiling experiments for a ala4/5 double knockout mutant showed multiple differences, including an increased abundance of sphingolipids hydroxyceramide (hCer) and glucosylceramide (GlcCer). These results support a model whereby the flippase activity of ALA4/5 impacts the homeostasis of both glycerolipids and sphingolipids, with this membrane remodeling activity being important for cellular expansion during growth in vegetative tissues.
8. The Allen lab (USDA/Danforth Center) investigated carbon partitioning in photosynthetic organisms including leaf and seed tissues of C3 and C4 plants. Tobacco leaves modified to produce high levels of lipids (collaboration with CSIRO Vanhercke; and Bates Lab WSU) appear to show compensatory production of other biomass components including starch. Analysis of fatty acid methyl esters (FAMEs), confocal microscopy with Nile red staining, and weight measurements after extraction and separation all confirm significant increases in lipid levels in leaves. On a carbon basis 40-90% of the carbon in lipid can be accounted for by reduction in starch levels. Changes in plant size were modest but may suggest a slight reduction in biomass production. 13CO2 was used to probe biosynthesis and turnover of biomass components. Methods to measure pool sizes of acyl-ACP in unlabeled and labeled form were developed and supported the increased biosynthetic rate of lipid production.
9. In separate studies by the Allen lab and in collaboration with Durrett Lab KSU, developing soybeans were probed with isotopes to interrogate the changes in metabolism and carbon partitioning during the development process, as green seeds lose any photosynthetic competency. Seeds have a reduction in total lipid accumulation late in development that is a consequence of carbon reallocation to other biomass components.
10. Studies at DDPSC (Allen lab) in collaboration the Moose Lab at UIUC on mutant lines of maize for PEPCK have been generated through transposon insertions by members of the Allen lab to study the role of minor decarboxylation pathways under different environments. We have performed RNAseq analysis on some lines to see changes in expression of other related C4 genes and to partially confirm reduction in pepck.
11. Biomass and labeling studies in *Chlamydomonas reinhardtii* wild type and a mutant line have been performed through a collaboration between Allen and Umen labs at DDPSC and are being refined to assess differences in metabolism that contribute to modest enhanced lipid production without requiring nitrogen deprivation.
12. Studies on the role of sorghum reproductive structures including paired spikelets and awns were performed with 14C, 13C isotopic analyses, RNAseq, microscopy analysis and experiments removing these organs on growing plants. These studies are a joint effort between the Kellogg Lab and Allen USDA Lab at DDPSC.
13. Studies by the Cushman lab reported Improved plant biomass and reproductive yields in Arabidopsis thaliana and Nicotiana sylvestris through transcriptional reprogramming of the auxin-signaling and response network by overexpression of a bHLH transcription factor (Lim et al., 2018).
14. One factor limiting starch production in developing rice grains is the heat lability of AGPase. The rice small subunit was engineered by the Okita lab to contain a QTC peptide motif, a major determinant of heat stability of the potato enzyme, near the N-terminus and L379F replacement. Addition of these two changes to the small subunit enhanced the heat stability properties of the enzyme. A manuscript describing these and other results have been submitted for publication.
15. Although starch synthesis during rice grain development was stimulated by the expression of a highly catalytically active bacterial AGPase, maximum carbon flow into starch was not close to being achieved. Transcriptome analysis performed by the Okita lab showed the activation of a starch binding domain containing protein (SBDCP). Biochemical studies showed that SBDCP binds to SSIIIa and inhibits catalytic activity noncompetitively. SSIIIa levels were also significantly lowered. Suppression of SBDCP expression by RNAi restored SSIIIa levels and mediated a small increase in grain weight. ADPglucose levels, however, remain elevated indicating that other processes prevent maximum flow into starch. A manuscript describing these and other results have been submitted for publication.
16. Past studies in the Okita lab demonstrated an unexpected interaction between the phosphorylase enzyme, Pho1, and PsaC, a major component of photosystem I. The interaction of Pho1 to PsaC suggests that Pho1 may control PSI activity and, in turn, plant growth. Direct evidence for such a relationship was obtained by measuring the *in vivo* redox changes in P700 using the LED-based flash spectrophotometer. The results showed that transgenic Pho1ΔL80 showed better photosystem 1 efficiency than wild-type plants and accounted for the increased growth rates of Pho1ΔL80 plants over wild-type.
17. In addition, studies in the Okita lab demonstrating the requirement for the RNA-binding protein, RBP-P, were completed and published as were studies showing that only a subset of the mRNAs were secreted extracellularly.

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

1. The Fritschi lab completed data analysis and published our research on carbon partitioning in N deficient maize.

2. In addition, diverse soybean genotypes were screened under high temperature conditions and genotypes contrasting in chlorophyll fluorescence have been selected for physiological studies and differential water use efficiency of soybean genotypes contrasting in carbon isotope discrimination was confirmed.
3. The Below Lab (IL-AES) developed an initial model to classify corn hybrids into how efficiently they produce grain under nitrogen or population stress environments. These results have been presented at the University of Illinois annual Agronomy Day to farmers and other agricultural professionals, and at an international meeting on nitrogen in the environment. Additionally, the results have been made available online at the university website serving a nationwide, as well as international audience. A section of this experiment has also been published in a scientific journal.
4. To understand the molecular mechanism how silicon improves the growth of soybean plants grown under water limiting conditions, the effects of silicate application on protein expression were examined in the Li lab. Soluble proteins from the leaves and roots of silicon-treated and control plants were separated by two-dimensional gel electrophoresis and differentially expressed proteins in response to silicate application under water deficit stress were identified by mass spectrometry. Proteins that shown differential expression in response to silicon application included reduced nicotinamide adenine dinucleotide phosphate-producing enzymes (nicotinamide adenine dinucleotide phosphate-dependent malic enzymes and 6-phosphogluconate dehydrogenase), detoxifying enzymes (peroxidases and aldehyde dehydrogenases), heat shock protein, pathogenesis-related proteins, proteins involved in the proteasome-dependent degradation pathway. These results indicate that silicate application could affect enzymes important for nicotinamide adenine dinucleotide phosphate production, detoxifying enzymes, proteins important for regulating protein folding/stability and protein abundance under water deficit stress, which may be attributable to improved growth of soybean plants grown under water limiting conditions.
5. The Harper lab performed an RNA-seq expression profiling analysis which was published that compared the heat-stress response in pollen from WT and a heat-sensitive mutant harboring a KO (knockout mutation) of cngc16 (cyclic nucleotide gated channel 16). This provides the first reference RNA-seq data set for a heat-stress response in pollen from the model plant Arabidopsis, and defines an extensive heat-stress dependent reprogramming of approximately 15% of the WT pollen transcriptome. In addition, it provides evidence that the heat-stress response in pollen has significant differences from vegetative tissues. For example, of the 89 transcription factors that showed a heat-stress dependent change in WT pollen, 84 (or 94%) failed to either be detected or show a similar response in aerial parts of Arabidopsis seedlings. In the context of understanding the heat-sensitivity of the cngc16 mutant, the mutant showed 2776 differences compared to the WT heat-stress response, with most of the largest magnitude changes having the potential to specifically impact cell walls or membrane dynamics.
6. The Harper lab also discovered that a loss-of-function of Arabidopsis thaliana Ca2+-ATPases 1, 2, and 7 result in plants with a salicylic acid dependent lesion-mimic phenotype and defects in pollen fitness. Imaging of cytosolic Ca2+ dynamics provided evidence that a loss of aca1/2/7 results in Ca2+-transients with larger magnitudes and duration, consistent with a role these Ca2+-pumps in restoring basal Ca2+ levels after a Ca2+-signal. Our results provide evidence that Ca2+-pumps ACA1, 2, and 7 function in the ER and together make important contributions to Ca2+- signaling dynamics in vegetative and reproductive tissues
7. Continued biomass and fruit production studies at the Cushman field site in Logandale, NV to examine the biomass production of three Opuntia (prickly pear cactus) species under three different irrigation regimes. We also expanded this work to include new field trials in collaboration with the USDA-ARS National Arid Land Plant Genetic Resource Unit (NALPGRU) in Parlier, CA. The national Opuntia germplasm collection is undergoing curation using molecular identifiers to characterize the genetic structure of the collection to resolve species designations and remove redundant germplasm accessions. To demonstrate the utility of Opuntia as a potential biofuel feedstock, we completed the analysis of fast pyrolysis of arid land biofuel feedstocks Opuntia ficus-indica (prickly pear cactus) and Grindelia squarrosa (Gumweed) (Cross et al., 2018). Species capable of growing in semi-arid and arid environments can serve as useful sources of hydrocarbons and carbonyl chemicals using rapid thermochemical conversion approaches for drop-in fuel blend stocks.
8. The Cushman lab also investigated the use of diverse perennial crops such as switchgrass, Miscanthus, Agave, and Opuntia for production on degraded and abandoned agricultural lands for the production of advanced biofuels particularly in semi-arid environments (Davis et al., 2018). Such feedstocks require less fertilizer input, have a greater potential for sequestration of carbon and soil carbon, reduce erosion, improve nutrient retention, and provide for greater ecosystem services and biodiversity on degraded agricultural lands than traditional annual crops.
9. Progress was made by the Cushman lab towards completing the transcriptome and genome sequence of Opuntia cochenillifera, a diploid, obligate CAM reference species for the Cactaceae. We also continued progress towards completing the transcriptome and genome sequencing of Opuntia ficus-indica, an agronomically important obligate CAM species (see above).
10. The Cushman lab developed a robust bioreporter sensor for in vivo, real-time detection of reactive oxygen species (ROS) based upon the expression of green and red fluorescent protein expression from ROS-responsive promoters to detect oxidative, salinity, and pathogen elicitor (flg22) stimuli (Lim et al., 2018).

Outputs: See attached list of Publications.

Plans for the Coming Year:

Objective 1:

The Benning lab will continue to explore the biotechnological application of PLIP1-based engineering of seed oil content and quality in Camelina. We are conducting a genetic suppressor screen in the PLIP3 cDNA over expression line to identify new components involved in the coordination of abiotic and biotic stress responses in plants. The reaction mechanism of FAD4 and the role of cofactors in this reaction will be completed and published. A first publication of the role of a rhomboid protease located in the chloroplast envelope membranes on phosphatidic acid biosynthesis will be completed. Inspired by results on the rhomboid protease, Benning's lab will 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.

The Kirchhoff lab will determine what kinases are involved in cyt b6f complex distribution and determine whether this is correlated with state transition (redistribution of LHCII). Furthermore, they will determine functional implications of cyt b6f reorganization. In addition, the lab will work to understand the role of protein kinase/phosphatases for ultrastructural thylakoid changes with focus on turnover of PSII-LHCII and will measure architectural membrane changes in thylakoid ion transporter/channel mutants and finalize the computer model and functional analysis.

Objective 2:

The Frischi lab will identify soybean accessions contrasting in light reaction characteristics in the USDA soybean germplasm collection based on genetic markers associated with chlorophyll fluorescence phenotypes and characterize the relationship between non-photochemical quenching and leaf pigments in soybean

The Sharkey lab intends to publish the data on characterization of G6P shunt enzymes and an analysis of the conditions that could lead to significant flux through the shunt (underway). Additionally, they will test ideas about the G6P shunt using transient expression of GPT2, plastidial starch phosphorylase, and other critical genes and use 13CO2 labeling to trace carbon through the G6P shunt and to identify a pool of carbon that is connected to the Calvin-Benson Cycle but that labels more slowly than most of the intermediates of the cycle (underway).

During the next year the Cousins lab intends to: perform field screens of $\delta^{13}C$ in Sorghum bioenergy mapping populations, determine the role of carbonic anhydrase in limiting C_4 photosynthesis under elevated temperature, and determine how specific amino acid residues influence the kinetic properties of phosphoenolpyruvate carboxylase, the enzyme catalyzing the first committed step of C_4 photosynthesis.

Objective 3:

In the Melis lab, the TLA principle could apply to other crop plants, promising to increase yields, while minimizing the space needed for cultivation. Higher density planting of grapevine, soybean or corn, among other crops, offers ancillary benefits, as these would achieve canopy closure more quickly and thus (i) minimize losses of soil moisture, (ii) lower the amount of fertilizer needed, as a smaller plot size with higher density of plants will minimize unwanted fertilizer runoff, and (iii) alleviate of the need to use herbicides, as quick and unbroken canopy closure will create the shading needed to prevent, or minimize, the growth of weeds. Efforts by the Melis lab will be made to extrapolate the findings in tobacco to the above-mentioned crop plants.

In the Roston, Stone and Bickford labs, work is underway to measure photosynthetic rates of antioxidant-grown plants and measure photosynthetic outputs (growth, yield, chlorophyll content) of antioxidant-grown plants. The groups intend to clone the antioxidant-producing biosynthetic pathway into E. coli to test production in aerobic environments.

In the next year, the Giroux lab will complete greenhouse yield trial of increased leaf and seed AGPase wheat and measure impact of increased AGPase upon plant yield components and test additional Rht alleles under greenhouse and field conditions. The Rht-B1b and Rht-D1b alleles both contain stop codons near the RHT N terminus. The lab is testing Rht alleles with stop codons located more distally. In addition, the Giroux lab will carry out yield trials of wheat populations having unique Rht alleles or increased leaf and seed AGPase.

During the next year the Gillaspy lab intends to refine observed changes in inositol pyrophosphates in mutants and transgenic plants, investigate missing enzymes responsible for InsP7 and InsP8 synthesis and breakdown, and use VIGS in cotton plants to suppress key inositol pyrophosphate pathway genes.

The Allen lab intends to: i) quantify metabolic fluxes in Chlamydomonas reinhardtii and vip1 mutant lines, ii) advance studies on fluxes in leaves augmented with genes to produce high amounts of lipid, iii) analyze altered maize lines with 13C isotopes to better understand the changes in C4 photosynthetic metabolism under altered environmental conditions, iv) further examine the changes in carbon partitioning in soybeans throughout development, v) publish work on the spikelet/awn role in sorghum development.

The Cushman lab will complete the introduction of a variety of synthetic gene circuits of the carboxylation and decarboxylation modules of CAM into Arabidopsis thaliana and complete the molecular, physiological, and photosynthetic analysis of selected transgenic lines.

The Okita lab intends to initiate gas exchange studies of Pho1ΔL80, Pho1, BMF136 (pho1 mutant) and TC65 (wildtype) plant lines, characterize plant lines expressing Pho1ΔL80 under the control of the stronger AGPase small subunit

promoter, and complete the study linking the membrane trafficking proteins, Rab5 and NSF, to RBP-P, and prepare and submit manuscript for publication.

Objective 4:
The Fritschi lab will continue growth chamber experiments to examine photosynthetic light reactions, carbohydrate dynamics, and chloroplast ultrastructure of contrasting soybean genotypes. Additionally, the lab will continue field experiments to characterize photosynthesis, morphology, and anatomy of soybean genotypes differing in water use efficiency.
The Below lab intends to repeat described experiments on corn hybrids to ascertain weather-year effects, investigate alternate models of hybrid characterization for yield production, present and publish the results at local, national, and international meetings.
To understand how silicon improves soybean photosynthesis under water limiting condition, the effects of silicon application on stabilizing chloroplast proteins and photosynthetic enzymes will be examined by the Li lab.
The Harper lab intends to test candidate stress-tolerance genes for their ability to improve heat-stress tolerance in pollen, determine the role of calcium second messengers in creating and propagating a long-distance signal from light-stress stimuli, develop and test new traits to improve the yield, oil quality, and stress tolerance in Camelina, an oil seed crop plant of potential economic importance for dry land agriculture, determine if a robust heat stress response in pollen involves the inhibition of a soluble inorganic pyrophosphatases (PPase) as part of a mechanism to slow-down cellular metabolism during a heat stress.
The Cushman lab will complete the transcriptome and genome sequencing of the Opuntia cochenillifera, a diploid, obligate CAM reference species and complete the analysis of the transcriptome of the Opuntia ficus-indica, an octoploid, obligate CAM species.

Impacts

Objective 1:
In the Benning lab, the discovery of the PLIP1 mediated chloroplast fatty acid export pathway provides a novel avenue for the engineering of high oil content in seed oil crops. Taking advantage of these findings, the lab has begun to develop technology to increase and modify seed oil content in Camelina and eventually canola. Target audiences will include biofuel feed stock producers as well as producers of vegetable oil for feed and human consumption. Findings on PLIP2 and 3 open up new ways to dissect the relationship between abiotic and biotic stress responses in plants. The Benning lab anticipates that understanding this relationship will provide new strategies in the future to generate more stress resistant plants in a changing environment. Likewise, the 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. The above-mentioned lipid tag and track method will be generally useful in studying lipid trafficking in plant tissues under different environmental conditions. It should be broadly applicable to address questions regarding lipid remodeling and trafficking in response to 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.**
Rebecca Roston (NE) has recently made a new collaboration with Nicole Buan (NE) and Julie Stone (NE) to investigate the role of an archaeal antioxidant on photosynthetic organisms. Application of the antioxidant to Arabidopsis resulted in plants that grew three times as large their control counterparts. As photosynthesis is a direct generator of plant biomass and redox stress, the lab will determine how the photosynthetic parameters are modified by an increasingly reduced environment. In addition to this work, they have also reviewed the lipid transport components required for production of photosynthetic membranes (LaBrant et al., 2018). In doing so, they identified some potential players and are now testing if they are involved through creation of DNA plasmids expressing fusion constructs of the gene and fluorescent markers.
Glenda Gillaspy (VT) has investigated the mechanisms that regulate sensing of energy status in plants. The response to low energy impacts photosynthate partitioning into biosynthetic pathways, related to Objective 3 of the project. Her lab has previously examined plant inositol kinase genes and the role they play in driving InsP6, InsP7, and InsP8 synthesis in plants, and how this relates to energy sensing and response. They have found that low energy transiently increases these molecules, suggesting that one or more of them plays a critical role in communicating energy status within plant cells. InsP6, InsP7, and InsP8 have also been recently implicated as controlling phosphate sensing in plants. The characterization of mutants defective in producing or maintaining InsP6, InsP7, and InsP8 levels in plants supports a key role for these molecules in phosphate sensing and lipid remodeling that likely occurs in the chloroplast. The lab suggests that future manipulation of key genes identified may be used to better control phosphate use in agriculture.
Adjusting the photosynthetic performance to environmental fluctuations is key for plant survival and is being investigated by the Kirchhoff lab. Central for these functional adjustments of photosynthetic energy transformation are architectural dynamics in thylakoid membranes. These structural dynamics occur on different levels ranging from

changes of the overall thylakoid membrane shape, to alterations on the mesoscopic level (organization of many pigment-protein complexes), to conformational changes of individual thylakoid membrane proteins. Knowledge of how thylakoid membranes change their structure on all these levels and identification of functional consequences of these structural alterations is required for an in-depth understanding of photosynthetic energy transformation in a challenging nature.

Objective 2:
The Fritschi lab characterized natural variation in chlorophyll fluorescence traits coupled with genome-wide association analyses to set the stage to develop a better understanding of the genetics underlying photosynthetic light reactions in soybean. Together with the loci of interest, the genotypes with extreme chlorophyll fluorescence phenotypes (i) are useful for fundamental studies of photosynthetic light reactions, and (ii) are prime candidates for germplasm improvement efforts targeting enhancements in photosynthetic light use efficiency. This research is related to the interests of U.S. soybean farmers, including the improvement of resource use efficiency and abiotic stress tolerance of soybean varieties. As such, this research complements projects funded by the United Soybean Board as well as the Missouri Soybean Merchandising Council.
In the Sharkey lab, recent studies indicate the gluconeogenic reactions of the Calvin-Benson cycle can be bypassed by exporting triose phosphate and reimporting glucose 6-phosphate, but this can stimulate the glucose-6-phosphate (G6P) shunt resulting in carbon loss during normal photosynthesis. This disadvantage may be balanced by efficient resupply of intermediates to the Calvin-Benson cycle in a stochastic environment. Understanding the rate and regulation of the bypass and shunt may result in discovery of ways to improve carbon metabolism of photosynthesis.
Acquisition and metabolism of carbon can determine how fast the energy of sunlight can be turned into photosynthetic products. The Cousins' lab is focusing on how carbon fixation is limited by the carboxylation of ribulose 1,5-bisphosphate by Rubisco, particularly in response to temperature. Secondly, rates of photosynthesis in both C3 and C4 plants can be limited by the availability of CO2 at the initial site of carboxylation. Therefore, understanding how leaf biochemical and anatomical traits influence the concentration of CO2 at the site of carboxylation has important impacts on the rates of photosynthesis.

Objective 3:
In the Melis lab, experimental evidence showed that decreasing, or truncating, the light-harvesting antenna size of the photosystems in the model plant Nicotiana tabacum (tobacco) helped to substantially increase the photosynthetic productivity and plant canopy biomass accumulation under high-density cultivation conditions. This Truncated Light-harvesting Antenna (TLA) technology can be applied to all agricultural plants, resulting in measurable improvement in crop yield per hectare of cultivated area. The work of the Melis lab showed, for the first time, that downregulation in the expression of the signal recognition particle 43 (SRP43) gene in tobacco conferred a truncated photosynthetic light-harvesting antenna (TLA property) and resulted in plants with a greater leaf-to-stem ratio, improved photosynthetic productivity and canopy biomass accumulation under high-density cultivation conditions.
Rebecca Roston (NE) in collaboration with Nicole Buan (NE), Julie Stone (NE) and Nate Bickford (NE) are investigating the role of archaeal antioxidants in hydroponic and aquaponics systems. Initial results from Arabidopsis indicated three-fold growth. However, in the hydroponic system the antioxidant remains oxidized for less than 24 hours. Thus far, a growth effect is still observed in a hydroponic system and will be investigated. In Arabidopsis, the growth effect seems to include total root and shoot biomass. Related work has led to additional funding for this group: **Roston, R., Stone, J., Buan N. IC-14532, Grant, "Capturing Archaeal biochemistry to build bigger botanical biomass", UNL Internal Funds, $149,065.00, Awarded. (start: July 1, 2018, end: June 30, 2020).**
In the Giroux lab, the long-term goal of the proposed research is to increase cereal agronomic yield by gaining a better understanding of how source and sink strength influence plant yield. We have focused primarily on leaf and seed starch by manipulating ADPglucose pyrophosphorylase (AGPase) levels in leaves and seeds. In doing so, we have found that increasing rice leaf and seed AGPase simultaneously increases plant productivity at moderate levels of soil fertility. We have also been examining whether wheat Reduced Height (Rht) mutations impact photosynthesis. Select Rht mutations in wheat create increased yield by decreasing plant height while increasing the number of fertile tillers per plant. Our project in the last year focused on examining the wheat semi-dwarfing allele Rht-B1b and how Rht mutations impact plant growth and development. We investigated the impact of Rht-B1b on: photosynthesis of plants grown under field conditions, carbon and nitrogen partitioning in major organs throughout development, and seed development throughout grain fill. From these experiments we hoped to observe key differences associated with the Rht semi-dwarfing alleles which can be used to help explain the mechanism by which Rht mutations increase plant productivity. Our goal is to identify ways to increase plant productivity whether by incorporation of modified Rht alleles into wheat varieties or selection for increased leaf and seed AGPase levels. We have 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. It is already well known that semi-dwarfing alleles increase productivity and economic return in many crops. Our hope is that our work will identify specific semi-dwarfing alleles that will still increase yield while having better agronomic properties than those currently used. Related funding has been awarded in support of these efforts: **Giroux, Martin. Wheat adaptation, yield, and growth**

effects of novel semi-dwarf alleles. USDA-AFRI. 2/1/17-1/31/19. $244,618 and **Giroux. Examining the Role of Nitrogen in Wheat Growth and Yield in Response to Increased Starch Biosynthesis. Montana Fertilizer Advisory Committee. 7/1/18-6/30/19. $34,000.**

Within the Gillaspy lab, the biochemistry of a new component of sugar-mediated signaling systems has been characterized. Knowledge has been gained on the lipid content changes in key mutants altered in energy/carbon status, as well as phosphate sensing. This can be used to guide improvements in crop plants for phosphate efficiency. The lab has gained understanding of how the inositol pyrophosphate signaling systems functions, which can be used in the future to engineer plants with improved phosphate efficiency and/or agricultural productivity. Related studies have helped secure funding including: **Securing our Food: A Translational Experience for Undergraduate Students in Plant Sciences, NIFA, $270,581, 01/01/2017- 12/21/2020, Co-PIs: William Frame, Hilary Mehl, Jacob Barney, and Laura Strawn** and **Collaborative Research: Modeling the Regulatory Network of InsP6 Signaling in Plants, NSF, $186,809,08/15/2016- 07/21/2019, Co-PIs: Imara Perera, Pablo Sobrado, Cranos Williams, Joel Ducoste.**

In addition to described impacts in Objective 4, the Harper lab examined the role of lipid flippases in lipid biogenesis and catabolism pathways.

The Allen lab is focusing on understanding carbon partitioning in different photosynthetic systems. Studies of mutant lines of maize for PEPCK in the Allen lab provide an important resource for us to explore the role of minor decarboxylation pathways in C4 metabolism which we hypothesize changes under different environments. Studies of biomass in leaves producing high lipid levels indicate that a leaf can fulfill both source and sink functions; with 30% biomass as lipid, coming partially at the expense of starch production. Studies in developing soybeans indicate reductions in lipid levels late during development that can be loosely correlated with increases in other biomass substituents and may provide carbon for these processes which remains under investigation. Studies on flux and carbon partitioning in C. reinhardtii provide an important way to assess carbon partitioning between growth and storage reserves because single celled algae will have merged source and sink functions. Studies on sorghum reproductive organs indicate a role for pedicellate spikelets in assimilating carbon that is transported to the sessile (seed-bearing) spikelet. Removal of the pedicellates resulted in a decrease in seed biomass of several percent and therefore is likely important to final crop yield. Recent activities related to work deciphering metabolic fluxes in photosynthetic systems and investigations in carbon partitioning in green seeds has led to funding by the National Institutes of Health: **JD Young, DK Allen: NIH Common Fund, "Tools for leveraging high-resolution MS detection of stable isotope enrichments to upgrade the information content of metabolomics datasets". 2018-2022.**

The long-term goal of Cushman lab research under Objective 3 is to improve plant productivity. As part of efforts to improve drought attenuation (or tolerance) in crops, the lab recently was able to successfully engineer increased tissue succulence in Arabidopsis thaliana (Lim et al., 2018). However, the overexpression of a bHLH transcription factor also resulted in significant increases in vegetative biomass and seed yield. This mechanistic basis of this 'large plant' phenotype involves a dramatic increase in auxin-mediated signaling and response network that promotes increased cell size and proliferation of lateral leaf and floral primordia. This transcriptional reprogramming was also possible in Nicotiana sylvestris suggesting that this innovative strategy might be applicable to improving biomass and reproductive yields in crops.

The sink strength of developing cereal grains is dictated by the conversion of transported photoassimilates into starch and protein, the major storage reserves. Current efforts in the Okita lab have been directed at understanding how starch and proteins are synthesized and stored in developing rice seeds. Specifically, we are interested in elucidating the role of the regulatory enzymes, ADPglucose pyrophosphorylase (AGPase) and starch phosphorylase 1 (Pho1) in starch biosynthesis and how storage protein synthesis is controlled by the localization of RNAs on distinct subdomains of the cortical endoplasmic reticulum.

Objective 4: Drought and high temperatures are the most important factors limiting crop yields around the world. In Missouri, drought is the most common reason for crop insurance payments to farmers. Thus, development of more drought and heat tolerant soybean varieties is critical for US and Missouri soybean farmers. The Fritschi lab previously screened many soybean genotypes for differences in carbon isotope discrimination and chlorophyll fluorescence. Based on these studies, we have identified genotypes that exhibit contrasting phenotypes, and thus, are suitable for physiological studies aimed at dissecting mechanisms underlying susceptibility/tolerance to water deficit stress and heat stress. These genotypes are also of interest for genetic studies and are being used to develop mapping populations and ultimately genetic markers associated with tolerance/susceptibility that can be employed by breeders in their efforts to develop more tolerant soybean cultivars. Given the impact of drought and heat stress on soybean yields and the climate conditions predicted for the future, this research is of great importance for U.S. soybean farmers. This research also complements ongoing projects funded by the United Soybean Board as well as the Missouri Soybean Merchandising Council.

Fred Below (IL) has investigated the responses of different corn hybrids to nutrient (nitrogen) and population density stresses. In corn, the primary focus of photosynthetic productivity is the commodity of grain yield. Developing strategies to overcome limitations to photosynthetic productivity is related to Objective 4 of the project. This study serves to identify 'Racehorse' hybrids, or hybrids that have greater than average yield increases with high-yield crop management, and 'Workhorse' hybrids, or hybrids with acceptable yields in a low fertility environment and tolerance to nitrogen loss. For 2017, 48 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). Exceptional yields were obtained in the unfertilized (check) plots, due to high residual N mineralized from soil organic matter, which in turn, led to a lesser yield response to N fertilization than seen in previous years. Averaged across all sites, plant density increases from 32,000 to 38,000 plants/acre contributed to a yield response of +10.4 bu/acre, but relatively little yield improvement (+3.1 bu/acre) was observed by further increasing to 44,000 plants/acre. On average, narrower rows were beneficial for increased yields at the highest plant population, however there was a wide range of yield variations in response to narrow rows among the commercial hybrids. In Champaign, yield responses to narrow rows ranged from a loss of 54.0 bu/acre to a gain of 58.2 bu/acre. Hybrids were ranked (1-10, with 10 indicating the greatest yield increase) by their yield responses to each parameter and 'Racehorse' and 'Workhorse' indices were then estimated using a multiple regression approach to characterize hybrids for their responsiveness to intensive crop management and/or tolerance to low N conditions, respectively. Check plot yield was most important in determining a hybrid's 'Workhorse' index, and yield responses to high N and row spacing were most important in determining its 'Racehorse' index. Commercial corn hybrids differ drastically in their ability to tolerate a low N environment and in their responses N fertilization, plant population, and row spacing. Understanding the agronomic factors that most influence grain yield for different corn genotypes will allow growers to most efficiently produce the greatest yields. 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.

Jiaxu Li (MS) has investigated the effects of silicate application on vegetative growth and photosynthetic biomass of soybean plants grown under water limiting conditions. This research is aligned to Objective 4 of the project - development of strategies to overcome limitations to photosynthetic productivity caused by developmental and environmental factors. The results from our study indicate that silicate application can improve the vegetative growth of soybean plants grown under water limiting conditions. Abiotic stresses decrease the growth by inhibiting the process of photosynthesis. Abiotic stresses such as drought and heat are likely to become severe problems with the predicted global warming. The intended long-term goal of our research is to improve photosynthetic productivity of crop plants under abiotic stress conditions. Our studies suggest that silicate supplementation may be a promising strategy for improving soybean growth under water limiting conditions. The following grant has been obtained as a result of our research activities: **Proposal Title: Role of a dehydration-stimulated phosphatase in regulating rice responses to drought and heat, Principal Investigator: Jiaxu Li, Funding Source: Mississippi Agricultural and Forestry Experiment Station, Award Amount: $42,600, Funding period: 00/01/18 – 12/31/18.**

The Harper lab investigated the role of calcium signaling under abiotic stress conditions that enable plants to survive, reproduce, and partition photosynthate into usable products such as seeds (Objectives 3 and 4). Arabidopsis was used as a model system to investigate the structure and biological functions of genes encoding calcium pumps (ACAs), calcium-dependent protein kinases (CPKs), lipid-stimulated protein kinases (PDKs), cyclic nucleotide gated channels (CNGCs), lipid flippases (ALAs), general regulatory factors called 14-3-3s, and Myb and CAMTA transcription factors. A key hypothesis guiding our research is that stress-triggered Ca2+ signals are used to coordinate the activity of multiple cellular processes, including changes in transcription and translation, metabolism, lipid signaling, membrane biogenesis, and regulation of cell wall biosynthesis. Projects are designed to provide novel and fundamental insights into how a network of cellular components are integrated into a robust stress-tolerance response.

The long-term goal of Cushman lab research under Objective 4 is to improve the water-use efficiency (WUE) of crops through increased tissue succulence (Lim et al., 2018). The net result of this approach is a significant increase in drought attenuation and improved WUE, which is defined as the ratio of carbon assimilation resulting in biomass accumulation to the rate of water lost due to transpiration. Improving the WUE of crops would allow them to be more resilient to drought under the more variable precipitation patterns (drought) that arise from global climate change or allow them to be grown with less water as agricultural water resources become limiting due to competition with municipal uses. Another approach is to introduce crassulacean acid metabolism (CAM) into C3 (or C4) photosynthesis crops to promote increased WUE and enable plants to use less water or to inhabit water-limited environments (Liu et al., 2018). As a first step towards engineered CAM, the lab has isolated and analyzed the subcellular localizations of 13 enzymes and regulatory proteins of the C4 metabolism cycle of CAM from the common ice plant in stably transformed Arabidopsis thaliana (Lim et al., 2018). In addition to improving plant growth, the overexpression of most carboxylation module components resulted in increased stomatal conductance and dawn/dusk titratable acidity as a measure of organic acid accumulation. In contrast, overexpression of some decarboxylation module components resulted in reduced stomatal conductance and titratable acidity. This study provides fundamental insights into the relative functional contributions of each of the individual components of the core C4-metabolism cycle of CAM and represents a critical first step in laying the foundation for CAM Bio-design.

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