Basic Information

Project No. and Title: NC1200: Regulation of Photosynthetic Processes
Period Covered: 11/01/2019 to 10/31/20
Date of Report: 1/14/2021
Annual Meeting Dates: 11/14/20 virtual by ZOOM

Participants

Presenters: Helmut Kirchhoff (kirchhh@wsu.edu) - Washington State; Rob Aiken (raiken@ksu.edu) - Kansas State University; Mike Giroux (mgiroux@montana.edu) – Montana State University; Doug Allen (Doug.Allen@ARS.USDA.GOV) - D. Danforth Plant Science Center St. Louis; John Cushman (jcushman@unr.edu) – University of Nevada; Glenda Gillaspy (gillaspy@vt.edu) -Virginia Tech University; Rebecca Roston (rroston@unl.edu) - Univ Nebraska Lincoln; Katarzyna Glowacka - (kglowacka2@unl.edu) - Univ Nebraska Lincoln; Asaph Cousins (acousins@wsu.edu) - Washington State University; Scott McAdams (smcadam@purdue.edu) – Purdue University; Anastasios Melis (melis@berkeley.edu) - University of California, Berkeley; Tom Sharkey (tsharkey@msu.edu) – Michigan State University; Berkley Walker, Michigan State University; Ru Zhang (ruzhang.danforthcenter@gmail.com) Donald Danforth Plant Science Center;
Other Attendees: Christoph Benning (benning@msu.edu) - Michigan State University; Jiaxu Li (jl305@bch.msstate.edu) – Mississippi State University; Fred Below (fbelow@illinois.edu) – University of Illinois Urbana/Champaign; July Stone (jstone@unl.edu) - Univ Nebraska Lincoln; S.V Krishna Jagadish (kjagadish@ksu.edu) – Kansas State University. John Erickson (John.Erickson@usda.gov) USDA. Qingyi Yu (qyu@ag.tamu.edu) – Texas A & M.

Brief Summary of Minutes of Annual Meeting

Scientific presentations (Presenters and scientific summary/goals)

Helmut Kirchhoff: The Kirchhoff lab aims to understand how photosynthetic thylakoid membranes modify their structure on different length scales in response to changing environmental conditions and how these architectural alterations determine the functionality and regulation of energy conversion.
Rob Aiken: The KS-AES environmental physiology group studies early season chilling effects on sorghum and terminal heat and water-deficit effects on both wheat and sorghum. Computer simulation models and remote sensing tools are applied to large-scale genetic variability trials, supporting genetic gain in crop productivity and stress tolerance.
Mike Giroux: The long-term goal of research conducted in the Giroux Lab is to increase cereal agronomic yield by gaining a better understanding of how source and sink strength influence plant yield.
Doug Allen: The Allen lab (MO-ARS) continues to study carbon partitioning from source to sink (leaves to seeds) in important crops including maize, sorghum, soybean, camelina, tobacco and algae for enhanced yield and biomass composition.
John Cushman: The Cushman lab (NV-AES) made significant progress towards engineering synthetic crassulacean acid metabolism (CAM) to improve water-use efficiency and plant productivity. They have also demonstrated the functional utility of engineering tissue succulence to improve drought and salinity tolerance in plants. The long-term goal of this research is to develop novel strategies to improve the water-use efficiency of photosynthate acquisition and partitioning under harsh environmental conditions with a particular emphasis on installing CAM into C3 (and C4) photosynthesis crops and the development of water-saving food/feed and bioenergy CAM crops.
Glenda Gillaspy: The Gillaspy lab (VA-AES) continues to study energy and sugar signaling mechanisms in a model species (Arabidopsis) and a cover crop (Pennycress) by examining the specific inositol phosphate signaling molecules called inositol pyrophosphates (PP-InsPs). This work has important implications for how plants sense and accumulate inorganic phosphate (Pi) which has an important relationship to both photosynthesis and photosynthate partitioning.
Rebecca Roston, Katarzyna Glowacka: The Roston, Glowacka, Buan and Stone labs (NE-AES) have generated a new collaboration to study the effects of archaeal antioxidants on photosynthesis including direct measurements of photosynthetic parameters and appropriate physiological measurements in multiple species. Their results indicate that this may be an effective method of increasing the speed and level at which non-photochemical quenching occurs in response to light. The long-term goal of this work is to improve understanding of optimizing photosynthesis by use of external or internal modification of redox potential.
Asaph Cousins: Growing world population will require an enhancement in the efficiency by which photosynthetic organisms capture, utilize, and store energy. Across terrestrial plants there are two enzymes that primarily control the initial photosynthetic reduction of atmospheric carbon dioxide (CO_2): ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and phospho*enol*pyruvate carboxylase (PEPC). In C_3 plants, atmospheric CO_2 passively diffuses into the leaf to the site of carboxylation by Rubisco. Alternatively, the initial carboxylation reaction in C_4 plants is catalyzed by PEPC, which uses bicarbonate (HCO_3^-) in the first committed and non-reversible reaction of

the CO_2 concentrating mechanism (CCM). Ultimately, C_4 photosynthesis uses compartmentalized Rubisco to assimilate the concentrated CO_2. Under many relevant environmental conditions (e.g. drought and high temperatures) the carboxylation reaction can limit photosynthesis in both C_3 and C_4 photosynthesis.
Scott McAdams: Photosynthesis is dependent on obtaining carbon dioxide from the atmosphere through stomata. Stomata are mobile valves on the surface of leaves that regulate the uptake of carbon dioxide from the atmosphere and the rate of water loss from the leaf via transpiration. The effect of stomatal aperture limiting photosynthesis is considerable under ideal conditions and particularly acute during drought. The McAdam lab investigates the regulation of stomata by the environment, through the lens of evolution, to understand how and why angiosperms are the most successful and highly productive group of land plants, upon which all agriculture is based.
Anastasios Melis: The Melis Lab contributed with the design of oligonucleotide fusion constructs, as protein overexpression vectors, that could be used in cyanobacteria for the overexpression of recalcitrant plant, animal, and human genes to levels reaching 20% of the total cell protein. The technology was successfully applied in the overexpression of transgenic terpene synthases from a variety of vascular plants in these photosynthetic microorganisms. Current work validated application of the "fusion constructs as protein overexpression vectors" and expanded the effort to include heterologous eukaryotic non-plant genes in the overexpression effort.
Tom Sharkey: Day respiration, or CO_2 release in the light, reduces the rate of photosynthesis, but the origin of the carbon has been unclear. This CO_2 release is often measured as the emission of $^{12}CO_2$ into a 99% $^{13}CO_2$ atmosphere. Last year they showed that this CO_2 originates from glucose 6-phosphate in the cytosol. This year they used stable isotope analysis to determine the source of this carbon, which can improve flux balance analyses of photosynthetic carbon metabolism.
Berkley Walker: Net photosynthetic capture can be approached as a mass balance between carbon uptake via the carboxylation reaction catalyzed by rubisco and carbon release from metabolism. The two main carbon releasing processes in an illuminated leaf are non-photorespiratory release (RL) and photorespiration. The biochemical drivers of RL are not well-resolved and many factors related to the flux of carbon through photorespiration downstream of rubisco oxygenation are unclear. Net photosynthetic capture also faces the challenge of supplying carbon to downstream metabolism despite wild fluctuations in absolute rates of rubisco carboxylation and oxygenation reactions. The Walker lab uses formal flux approaches, in vivo measurements of gas exchange, and in vitro biochemistry to understand the metabolic drivers behind photosynthetic capture, with a special emphasis on the CO2 releasing processes like photorespiration and RL. They are also interested in how carbon fixation and photorespiration can supply intermediates to meet the demands of downstream metabolism despite large temporal fluctuations in the rates of both.
Ru Zhang: The Zhang lab studies how photosynthesis responds to temperatures by using both green algae and land plants as models. Their ultimate goal is to engineer more efficient and robust photosynthesis under stressful temperatures for improved agricultural production and biomass accumulation.

Business Meeting Summary

John Erickson USDA-NIFA summarized developments at NIFA and their move to Kansas City, as well as new funding opportunities. An effort will be made to keep funding deadlines and timelines more regular for future years. Plant Physiology maximum award sizes have gone up to $750,000. New seed projects for new investigators are coming online. Limits have been raised to $300k. Big initiatives with NSF and joint funding for AI are in preparation.
New Member Qingyi Yu was introduced. She is working at Texas A &M on CAM and C4 photosynthesis in pineapple and C4 grasses. She was unanimously approved by email vote following the meeting.
Renewal of NC1200 in 2021. Deadlines were discussed. Rob Aiken volunteered as lead organizer, Christoph Benning will stay on as Admin Advisor and assist. Proposed lead writers for the different objectives are: Objective 1, Rebecca Roston; Objective 2, Asaph Cousins; Objective 3 Tasios Melis; Objective 4, Krishna Jagadish and John Cushman.
The 2021 Meeting will be held in Nebraska organized by Rebecca Roston.
The 2022 Meeting will be held in Reno organized by John Cushman.

Accomplishments

Activities in 2020 are summarized under the different Objectives

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

- We studied the subcellular location of chloroplast lipases LPPγ, ε1, ε2. LPPγ appears to be associated with the outer envelope membrane while LPPε1 with the inner and possibly outer envelope membranes. LPPε2 is located at the inner envelope or the thylakoid membranes. An lppγε1 double mutant shows reduced growth that can be restored by introducing the full-length LPPε1 but not the full-length LPPε2 cDNAs. Hence the two isoforms have different functions or are located in different chloroplast sub-compartments. An LPPε2 cDNA with a partially

truncated transit peptide does complement the lppγε1 double mutant corroborating different locations of the two isoforms within the chloroplast.

- We have shown that RBL10 and integral membrane protease affecting chloroplast lipid trafficking occurs in a large complex in the inner envelope membrane. We tentatively identified by CoIP and split ubiquitin yeast two hybrid assays potential interactors of RBL10. One of them is ACP4 involved in fatty acid metabolism in chloroplasts that we are further investigating. We were able to produce various tagged versions of RBL10 in E. coli and are in the process of developing a protease assay for RBL10. We determined the likely topology of RBL10 in the inner envelope membrane. RBL10 has a seventh transmembrane domain at its C-terminus. The N-terminus is located on the stroma side of the inner envelope membrane. We have raised antibodies against RBL10.
- We continued the analysis of the reaction mechanism, activation, and function of the unusual FAD4 desaturase of Arabidopsis. FAD4 is responsible for the formation of a phosphatidylglycerol species in chloroplasts that contains a 16:1 delta 3 trans fatty acid at its sn-2 position. We published the identification of a new protein cofactor, a specific peroxiredoxin, required for the activity of FAD4 in vivo and in vitro. We were able to reconstitute the desaturase and its cofactor in yeast microsomes. Specific cysteine residues in FAD4 and peroxiredoxin were identified that are essential for activation and activity of FAD4. We are now exploring how this peroxiredoxin affects FAD4 activity. We hypothesize that this protein links the production of the unusual lipid species with the redox state of the chloroplast, which is likely affected by abiotic stress conditions, to protect the photosynthetic machinery. As a next step we are investigating lipid turnover in transgenic lines and mutants with varying abundance of the peroxiredoxin and the unusual lipid species.
- We finalized and published our studies on lipid-protein interactions in photosynthetic membranes (see JBC publication). For this project we established over several years a proteoliposomes technique that incorporates the isolated light harvesting complex II (LHCII) protein from thylakoid membranes into large unilamellar vesicles (LUVs) with a defined lipid composition. We found that the incorporation of the abundant non-bilayer lipid monogalactosyldiacylglycerol (MGDG) into proteoliposomes leads to energy dissipation in LHCII. This is the first report showing that this special lipid type controls the functionality of a photosynthetic thylakoid membrane protein. We formulated the hypothesis that the MGDG-induced energy quenching is induced by a conformational change in LHCII caused by alterations in the (hydrostatic) lateral membrane pressure in the hydrophobic part of the lipid bilayer.
- We finalized and published a project on long-range electron transport in photosynthetic membranes. By using mutants with different diameters of the stacked grana region leading to a longer or short diffusion distance between photosystem II (in grana) and photosystem I (in unstacked thylakoid domains) we could show that plastocyanin and not plastoquinone is responsible for long-range charge transfer. This solved a long-standing question in photosynthesis research.
- We established our ultrastructural thylakoid analysis pipeline that allows measurements of quantitative thylakoid parameters in leaf samples. This procedure combines optimized leaf fixation procedures, electron microscopy and computer-based image analysis.
- Finally, we made significant progress in establishing immunogold labeling of thylakoid protein complexes combined with transmission electron microscopy to examine dynamics of lateral protein distributions between stacked and unstacked thylakoid domains.

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

- We used membrane inlet mass spectrometry (MIMS) to measure Rubisco and PEPC kinetic properties, concentration changes in their substrates (HCO_3^-, CO_2, O_2) and isotope fractionation. Recently, we measured the temperature response of Rubisco kinetics and isotope discrimination from *Oryza sativa* to test if isotopic carbon discrimination is correlated to changes in the elementary rate constants. However, Rubisco discrimination was constant with temperature suggesting that kinetic changes in this species were not associated with changes to elementary rate constants but were instead associated with continual deactivation of the enzyme with temperature. However, this may not be true for Rubisco enzymes from C_4 plants that have different kinetic properties. Additionally, we modified specific amino acid residues in PEPC to determine their influence on HCO_3^- kinetics.
- We collected data on photosynthetic gas exchange and light reactions on soybean genotypes released over 80 years of breeding. Initial analyses indicate that light reactions changed with year of cultivar release, but this was not the case for leaf-level photosynthetic rates.
- Stable isotope analysis revealed that the release of CO_2 during photosynthesis (day respiration) could be accounted for by a cytosolic glucose-6-phosphate shunt recycling carbon back to the Calvin Benson cycle. The shunt likely operates at about 5% of the rate of carbon fixation, according to measurements made this year. In addition, unlabeled carbon enters metabolism, likely through hexokinase or fructokinase, suppressing the degree of label in cytosolic carbon pools and accounting for the fact that day respiration is significantly unlabeled. These experiments also showed that the degree of label in isoprene emitted from leaves of poplar is indistinguishable from the degree of label in Calvin Benson cycle intermediates, allowing isoprene labeling to be used as a non-destructive measure for studying labeling of the Calvin Benson cycle.

- We determined that the biochemical source of non-photorespiratory release of CO_2 at about 10% the rate of net carbon uptake from rubisco, is not from tricarboxylic acid cycle related reactions as commonly assumed. This was determined by combining gas exchange measurements with ^{13}C-Metabolic Flux Analysis (^{13}C-MFA) through central metabolism. This flux analysis supports that alternative flux through the oxidative pentose phosphate pathway in the light explains this CO_2 release.
- We found that the pool sizes and fluxes of central metabolism are surprisingly resilient to large (10-fold) changes of flux through photorespiration and that glycine plays a large role in determining how quickly net photosynthesis reaches a new steady-state following a change in photorespiratory rates. This was determined from 13C-MFA analysis, quantitative metabolomic measurements and gas exchange experiments performed under different photorespiratory conditions.
- We have continued our work conclusively demonstrating that H_2O_2 can drive alternative decarboxylation reactions that reduce the carbon recycling efficiency of photorespiration and that under ambient temperatures catalase protects against this photorespiratory inefficiency. This is demonstrated through a combination of mutant analysis, in vivo gas exchange and metabolomics. These findings set up our future work to determine if these reactions reduce the efficiency of photorespiration in wild type plants at elevated temperatures.

Objective 3. Mechanisms regulating photosynthate partitioning

- We nearly completed flux maps on algae grown autotrophically and mixotrophically to characterize the difference in carbon partitioning and the utilization of acetate as a carbon source. Generally, algae grow fastest mixotrophically, and growth exclusively on acetate without light (i.e. heterotrophically) is slow. We used isotopes to assess the extent of acetate carbon repurposing for growth and biomass production. Our results indicate use of acetate for production of organic acids and energy through the TCA cycle and as carbon skeletons for amino acids; however interestingly gluconeogenesis is not significant. The Calvin Benson Cycle maintains an important role even in the presence of acetate as indicated by multiple isotopic labeling experiments and metabolic flux analysis.
- We continued flux studies in tobacco lines that are overexpressing multiple genes and that result in levels of 30% lipid or more as a component of biomass. Our work describes that in mature leaves (i.e. photosynthetically competent but with little expansion), that lipid increases dramatically as a tradeoff with starch. Starch can accumulate non-transiently as leaves age resulting in tobacco (and some other crops) with a 'sink' pool of starch that is not crucial to diurnal metabolism. Labeling experiments up to 5 hours were focused specifically on the starch/lipid tradeoff and suggested somewhat unexpectedly that significant unlabeled starch is produced at the same time as labeled starch from $^{13}CO_2$ provision, suggesting a source of starch production in other cell types.
- Other studies in the lab are describing the impact of altering C4 photosynthetic processes and the contribution of reproductive tissues to the carbon economy in developing grains and seeds.
- We reported on the biomass and seed oil productivity of false flax (*Camelina sativa*) under irrigated conditions in the desert Southwest and characterized a *C. sativa* mutant defective in seed coat mucilage.
- We obtained highly accurate vegetative and fruit biomass production results for life cycle assessment (LCA) and life cycle costing (LCC) analyses for bioenergy production modeling from prickly pear cactus (*Opuntia* spp.).
- Starch is an important metabolite in both source and sink metabolism. In rice and wheat, we have shown that increased leaf and seed starch biosynthetic rates increase plant growth rates and productivity under controlled conditions. We conducted a screen of wheat varieties and landraces and found that over the last 100 years that flag leaf starch has increased as has wheat yield. We also discovered landraces that are sources of much higher flag leaf starch levels. We are now analyzing recombinant inbred line populations created by crossing a hard red spring wheat with high leaf starch landraces. Each of the high starch landraces selected has up regulation of starch biosynthetic genes as measured by RNAseq. The populations were grown in the field in 2020 and we measured agronomic traits and yield on all lines. We are now measuring leaf starch on all lines using flag leaves collected from this summer's yield trial.
- We published results showing that a loss-of-function of two lipid flippases, ALA4 and ALA5, alters the homeostasis of specific glycerolipids and sphingolipids and is important for cellular expansion during vegetative growth. We discovered an *ala4/5* suppressor mutation that revealed an enzyme feature in a different subgroup of flippases that can be altered to enable them to rescue the growth defects associated with an *ala4/5* dwarf.
- Expression of human proteins in photosynthetic organisms have been described in the literature with mixed success due to very low levels of such recombinant protein accumulation. In plants and microalgae, most of the research entailed a heterologous transformation of the chloroplast, but transformant cells failed to accumulate the desired recombinant proteins in significant quantities. Our current work applied the "fusion constructs as protein overexpression vectors" and provided evidence for the overexpression in cyanobacteria, at the protein level, human-origin biopharmaceutical and biotherapeutic proteins. Proof-of-concept was provided for the design and reduction to practice of interferon α-2 (IFN). IFN is a member of the Type I interferon cytokine family, well-known for its antiviral and anti-proliferative functions. Fusion construct formulations enabled accumulation in cyanobacteria of IFN up to 12% of total cellular protein in soluble form. In addition, the work reported on the isolation and purification

of the fusion IFN protein and preliminary verification of its antiviral activity. Combining the expression and purification protocols developed here, it is possible to produce fairly large quantities of interferon and other biopharmaceutical and biotherapeutic proteins in these photosynthetic microorganisms, generated from sunlight, CO_2, and H_2O. Thus, the work showed that recombinant protein overexpression in cyanobacteria by the "fusion constructs as protein overexpression vectors approach is not limited to genes of the isoprenoid biosynthetic pathway from plants, but it does include human and animal origin eukaryotic proteins.

- We advanced our understanding of application of antioxidants in terms of amounts, application styles, rates and time of day, and species. Current species showing growth benefits include *Arabidopsis thaliana*, *Ocimum basilicum*, *Nicotiana tabacum*, and *Cannabis sativa*. Based on these results, we hypothesize the effect of generating a reducing growth environment is broadly beneficial to a wide range of organisms.
- We furthered our analysis of the photosynthetic effects of antioxidant application. The reduction of non-photochemical quenching is dependent on the presence of zeaxanthin epoxidase and PsbS and appear to be highly reproducible in *A. thaliana*. Interestingly, this implies a redox-based regulation of non-photochemical quenching which we intend to pursue further by measuring the effects of antioxidant application on the proton gradient and use of additional sensors of the photosynthetic environment. Because of the drop in NPQ, we do see a loss in adaptation to high light environments, implying that this treatment might be best for plants growing in low-light conditions. Similar reproducibility has yet to be seen in *N. tabacum*.
- A major question is whether the antioxidant is internalized into cells before it affects photosynthesis. We have made multiple types of measurements, which have conflicting results. Over the next year, a solid effort will be to resolve and understand these conflicts and clarify the timing of the effect. To aid us, we have developed three gene sets that are successful in producing the archaeal antioxidant in *E. coli* during aerobic growth. This sets us up to move one or more of these systems into plants, where antioxidant will be produced inside the cell types of our choice.
- We study the triose phosphate utilization (TPU) limitation of photosynthesis, the situation where photosynthesis can go faster than the plant can process its products. We showed that when plants are put into conditions that cause TPU, carbon metabolism and electron transport are regulated to match the lowered demand. Within a short time, the reduced rates of carbon metabolism results in a longer-term reduction in carbon fixation capacity. Photosynthesis therefore begins to look like it is limited by rubisco capacity even though it was limited TPU capacity that leads to reduced rubisco capacity. This is why plants rarely appear TPU limited but very frequently are close to TPU limitation.

Objective 4: Developmental and Environmental Limitations to Photosynthesis.

- Field studies identified similar upper limits to water productivity for above-ground biomass (5.6 kg m^{-3}) and grain yield formation (5.9 kg m^{-3}) of corn and grain sorghum. Water use during grain filling accounted for the most variation in crop productivity in the semi-arid cropping system. Insights into the impact of high day temperature stress during flowering and high night temperature interactions with VPD impacting crop yields were highlighted. In addition, we show a significant reduction in starch leading to an increase in protein and lipid accumulation in wheat grains, which can potentially lead to poor quality bread and other food products. Newly developed sorghum hybrids were shown to provide partial tolerance to early-stage chilling tolerance under field conditions. We quantified the impacts of high temperature stress under controlled environment and field conditions. These studies improved our understanding of physiological and biochemical basis of high temperature tolerance in sorghum and wheat.
- Forty-four commercial soybean varieties were evaluated across three north to south locations in Illinois with different inherent soil fertility and weather. Greatest yields were obtained by growing the fullest maturity group cultivar suitable for each site. Sixteen varieties grown at all sites were used to develop a yield-response to management classification model. Varieties with above-average yield increases in response to added fertility and foliar protection were considered 'Offensive'. In contrast, varieties demonstrating exceptional yield without added fertility or foliar protection were considered 'Defensive'. Varieties with above-average yield responses to agronomic management and good yield under the less intensive management conditions were considered 'Resilient'. These classifications support growers in selecting the ideal variety for their soybean management system.
- We grew corn at three sites with various forms of phosphorus and sulfur fertilizers with the goal of increasing phosphorus fertilizer use in maximizing yields. Fall-applied MicroEssentials S10 (a co-granulated fertilizer providing sulfur as both sulfate and elemental sulfur) increased soil P availability in the top 6" of soil because the oxidation of the elemental sulfur generated sulfuric acid which fostered hydrolysis of bound P, while preventing new binding of P to the soil calcium.
- In soybean, pre-plant applications of urea induced greater early-season vegetative and leaf growth, which can increase photosynthetic capture, but this N application also reduced symbiotic nodule formation over the growing season. Conversely, late-season N applications by sub-surface fertigation or by banding down the center row increased yield because these applications did not interfere with nodule development or biological N_2 fixation.

- Planting higher populations of soybean improved yield under late-planted conditions, due to greater light interception. Protecting the leaf canopy with a fungicide plus insecticide application increased yield of late-planted soybean, regardless of planting population.
- A new and rapidly expanding development in agricultural production systems is to use biostimulants to maximize nutrient availability from the soil and to improve the uptake efficiency or fertilizers. In 2019 we banded phosphorus-solubilizing bacteria (trade name iNvigorate), in-furrow at planting of corn and were able to increase yields, as well as nitrogen, zinc, and phosphorus uptake by the plants.
- We demonstrated the practical use of tissue succulence engineering, which results in improved water-deficit and salinity stress tolerance in *Arabidopsis thaliana*. Build upon a previous CAM Biodesign foundation by installing multi-gene circuits comprising the carboxylation and decarboxylation modules of CAM and the complete core C_4 metabolism circuit of CAM in *A. thaliana*. Other projects advanced progress towards stable transformation systems for the common ice plant (*Mesembryanthemum crystallinum*) and prickly pear cactus (*Opuntia ficus-indica*).
- We have leveraged genomic and transcriptomic resources from *M. crystallinum* to explore phylogenetic relationships with the *Amaranthaceae* and betalain pigment production in the Caryophyllales. We characterized the light-responsive gene atlas in *Kalanchoë fedtschenkoi*. Lastly, we reported on the genome sequencing of a halophytic green alga (*Dunaliella salina*).
- We have developed and genotyped a biparental mapping population segregating for water use efficiency and have phenotyped the population for photosynthetic gas exchange. Preliminary analyses indicate several QTL based on a single location of phenotypic data.
- We reported on the effects of elevated air temperature on the ultrastructure of chloroplasts and light reaction characteristics in selected soybean genotypes.
- We used machine learning approaches to better predict soybean yield from in-season UAV-based image acquisition
- Several Arabidopsis plant lines with modified levels of PP-InsP synthesis and degradation enzymes have been constructed and characterized. PP-InsP profiling indicates we have lines with increases or decreases in PP-InsPs.
- Analysis of plants with modified PP-InsPs levels indicates levels of PP-InsPs control Pi accumulation within plants.
- A synthetic biology approach has been used to decrease PP-InsPs in Arabidopsis and also within a cover crop, pennycress.
- We made improvements in a new reporter that allows for more confident imaging of Ca^{2+} signals in response to biotic and abiotic stimuli. This reporter provides a ratiometric measurement that allows more accurate comparisons of signal strength between different tissues, cell types, and subcellular locations.
- Research continued into the phenotype of a plant harboring a triple knockout of *Arabidopsis thaliana* Ca^{2+}-ATPases 1, 2, and 7. Imaging of cytosolic Ca^{2+} dynamics triggered through blue-light receptors (Phototropins) or a pathogen elicitor provided evidence that a loss of ACA1/2/7 results in Ca^{2+}-transients with larger magnitudes and duration, consistent with a role these Ca^{2+}-pumps in restoring basal Ca^{2+} levels after a Ca^{2+}-signal. Our results provide evidence that Ca^{2+}-pumps ACA1, 2, and 7 function in the ER and together make important contributions to Ca^{2+}-signaling dynamics in both vegetative and reproductive tissues.
- In order to better understand the molecular mechanism how silicon improves the growth of soybean plants grown under water limiting conditions, the effects of silicate application on chloroplast protein expression were examined. The results suggest that silicon application could affect enzymes important for photosynthesis and stabilize photosynthetic proteins and enzymes under water limiting conditions.
- We conducted experiments to investigate which physiological adaptations allow species from the aquatic fern family *Marsileaceae* (water clovers) to be the only family of non-angiosperm plant to have evolved high rates of photosynthesis, rivaling those observed in angiosperms. We discovered convergent evolution in a novel physiological capacity of stomata that was critical for the evolution of high photosynthetic rates in angiosperms and *Marsileaceae*: the capacity of stomata to open by lateral displacement into neighboring epidermal cells. This anatomical adaptation combined with a high stomatal density in species from both groups allows leaves to attain very high rates of gas exchange per unit area of leaf. These results have considerable implications for understanding the limitations placed on maximum photosynthetic rate by stomatal anatomy and physiology. Furthermore, comparisons between *Marsileaceae* and angiosperms suggests that the hormone abscisic acid (ABA), which closes stomata in angiosperms during drought, was essential for plants of this group to dominate terrestrial ecosystems.
- Further investigation into the stomatal physiology of *Marsileaceae* has revealed that this group of plants recently evolved a stomatal response to low fluences of blue light. Blue light accelerates stomatal opening in angiosperms independently of photosynthesis and is believed to be responsible for driving rapid stomatal responses to changes in light environment. The common ancestor of ferns have lost a stomatal response to blue light, our work in *Marsileaceae* have found that this response has secondarily evolved in this clade of highly productive ferns. We found that blue light stomatal responses in this group of plants is essential for rapid stomatal responses to both increases and decreases in the fluence of light, which might explain why angiosperms have such rapid stomatal responses compared to other plants, particularly a rapid closing response when light levels and photosynthesis declines.

- Investigation into the role of stomatal closure and cuticular conductance on plant water loss rates during leaf development and drought continues. We have found that the cuticle contributes to considerable water loss from a leaf during leaf expansion that that leaf photosynthesis does not begin until leaves have expanded sufficiently to tear the cuticle covering above developing stomata. In addition, we have found that the lethal threshold water potential at which sunflower plants can tolerate drought (when embolism in the xylem forms) can be altered by osmotic adjustment, suggesting that plant survival during water deficit can be prolonged by osmotic adjustment and a shift in the vulnerability of xylem to embolism.
- We characterized how the C4 model plant *Setaria viridis* responds to high light or high temperatures at photosynthetic, ultrastructural, and transcriptomic levels.
- We performed omics using the model green alga *Chlamydomonas reinhardtii* to investigate the system-wide changes of cell physiologies, transcripts, proteins and pathways in response to moderate and acute heat stress.
- We have characterized a few genes which were identified from our genome-wide mutant screen under high temperatures and have potential roles in algal heat tolerance.
- We investigated the possible mechanisms of cold and salt tolerance of the Antarctic green alga *Chlamydomonas* sp. UWO241 (UWO241) which has sustained cyclic electron flow (CEF) around photosystem I (PSI).

Outputs

See Publications below.

Plans for the Coming Year

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

- Continue the analysis of the function and localization of the three LPP proteins in Arabidopsis chloroplasts. We will focus our analysis of RBL10 on determining protease activity of the recombinant protein and will investigate whether the observed ACP4/RBL10 interaction has biological relevance. We will also complete our functional study of PLIP2 an PLIP3 proteins.
- Investigate growth and photosynthesis of transgenic lines and mutants altered in the amount of PG containing 16:1 delta 3 trans fatty acid to explore its hypothesized role in protection of the photosynthetic membrane. We will also determine if lipid turnover is altered in these lines.
- Continue our studies on cytochrome b6f redistribution by biochemical techniques and immunogold labeling of cytochrome subunits in thin sections followed by electron microscopic analysis.
- Continue the work on the thylakoid ultrastructure in thylakoid ion transport/channels mutants to identify their role in architectural dynamics.
- Establish dynamic coarse-grain computer models of thylakoid membranes to understand structure-function relationship for electron transport and light harvesting.

Objective 2. Identify strategies to modify biochemical and regulatory factors that impact the photosynthetic

- Determine kinetic parameters driving the temperature response of Rubisco kinetics from two diverse C_4 species.
- Determine if substitutions of specific amino acid residues in C4 isoforms of PEPC drive variation in kinetic properties
- Continue characterization of photosynthetic gas exchange in soybean biparental mapping population and identify QTL associated with photosynthetic gas exchange.
- Determine if glycine plays a necessary role in the flexibility and resilience of photorespiration to changes in photorespiratory rate imposed by changing atmospheric CO_2/O_2 concentrations and even variation in light intensities.
- Explore how photorespiration adapts to elevated temperatures and if there is evidence that alternative decarboxylation reactions during photorespiration factor into this adaptation.
- Characterize the kinetics of photorespiratory genes to finish our reaction-kinetic model of photorespiration
- Use various modeling approaches to understand how the structure of the C3 cycle and photorespiration can contribute to metabolic flexibility

Objective 3. Mechanisms regulating photosynthate partitioning

- Complete documentation of the differences in metabolic fluxes in algal central metabolism under auto- or mixotrophic metabolism
- Describe changes in maize C4 photosynthesis under altered conditions
- Investigate the tradeoff of lipid and protein in green oilseeds
- Report on the results of biomass productivity field trials, cladode area index (CAI) models for multiple accessions of Opuntia, life cycle assessment (LCA) and life cycle costing (LCC) analyses related to bioenergy production from *O. ficus-indica*, and the determination of the causative agents of Opuntia stunting disease.
- Map leaf starch in two RIL populations.
- Determine to what degree wheat productivity might be impacted by selecting for increased leaf starch.

- Compare the activity of isoprenoid biosynthetic enzymes in their native versus fusion construct formulations.
- Elucidate the mechanism upon which the “fusion constructs as protein overexpression vectors” approach enables this breakthrough technology.
- Explore how soluble redox reagents are modifying non-photochemical quenching capacity of *A. thaliana*
- Determine if the photosynthetic effects on A. thaliana are generalizable to other species.
- Explore the relevance of internalization of antioxidants.
- Determine if the loss of the G6P shunt eliminates the slow labeling component of the Calvin Benson cycle using loss of function mutants for cytosolic glucose-6-phosphate dehydrogenase
- Determine how loss of function of hexokinase and fructokinase affects carbon recycling
- Measure plant growth, yield, and resilience of plants lacking the G6P shunt
- Use metabolomics and rubisco assays to determine how photosynthesis is regulated when first exposed to TPU-limiting conditions

Objective 4: Developmental and Environmental Limitations to Photosynthesis.

- Screen sorghum mutants (cv. Tx642) for truncated light-harvesting apparatus, using remote sensing tools and protocols previously developed.
- Report water deficit effects on leaf photosynthetic activity and primary productivity of *Sorghum bicolor* and *Silphium integrifolium*
- Determine the role of functional stay-green as a means to reduce the negative impact on carbon balance (photosynthesis versus respiration) under high nighttime temperature
- Identify genomic regions controlling high nighttime temperature tolerance to optimize carbon balance in winter wheat
- Continue to study effects of fertilizers and foliar protectants on crop photosynthesis and productivity.
- Investigate alternate models of hybrid characterization for yield production.
- Explore biologicals as an agronomic management strategy to increase nutrient use and/or photosynthetic efficiency.
- Understand how changes in row spacing interact with other management practices (such as plant population, better placement of fertilizers, foliar protection, biologicals) to enhance light interception and/or the maintenance of leaf health
- Continue work on transcriptome and genome sequencing of two obligate CAM species: *O. cochenillifera* (diploid) and *O. ficus-indica* (octoploid) and associated metabolomic profiling under drought conditions.
- Report on the optimization of synthetic CAM potentially co-engineered with tissue succulence in A. thaliana.
- Rontinue our characterization of the phenotypic diversity within the USDA-ARS germplasm collection of Teff (*Eragrostis tef*) and drought-tolerance accessions of *E. tef*.
- Continue physiological characterization of obsolete and modern soybean cultivars to establish mechanisms that contributed to yield increases in soybean.
- Investigate the responsiveness of stomata to changing environmental conditions in soybean genotypes known to contrast in water use efficiency.
- Apply UAV-based approaches to estimate physiological and growth responses of soybean.
- Examine phosphate storage response gene expression, measure Pi uptake, and other physiological parameters in Arabidopsis transgenics with altered PP-InsP levels.
- Construct Arabidopsis and pennycress containing an inducible synthetic gene to increase Pi accumulation and reduce negative growth impacts.
- Measure total mass Pi balance in transgenics in collaboration with engineers.
- Test candidate genes for their ability to improve heat-stress tolerance in pollen.
- Determine how Ca^{2+}signals are modified by regulation of Ca^{2+}pumps and channels.
- Investigate the role of lipid flippases in promoting cellular expansion and plant growth.
- Generate overexpression lines of chloroplastic fructose-1,6-bisphosphatase and test stress tolerance profiles of gene-overexpressing lines.
- Continue to investigate the underlying developmental differences that allow stomata to open by lateral displacement into epidermal cells and attain very high rates of gas exchange.
- Explore the role of the hormone ABA on limiting gas exchange during drought in angiosperms; and how the evolution of the stomatal response to this hormone led to the ecological success of angiosperms.
- Continue to investigate the determinants of the lethal thresholds for plants during drought and how these might be altered to prolong productivity during periods of water deficit.
- Complete the algal omics analysis and mutant screen results, combine the datasets to identify genes and pathways that are important for heat tolerance in algae and land plants.
- Understand the regulation of NPQ, CEF, and Rubisco activase in C4 model plant *Setaria viridis.*

- Investigate the regulation of CEF in the Antarctic green alga Chlamydomonas sp. UWO241 and the model green alga Chlamydomonas.

Impacts

- The analysis of drought and high temperature stress metrics as an indication of stress avoidance traits will guide development of new cultivars with enhanced drought and heat tolerance. High temperature tolerant donors, physiological traits and associated genomic regions identified will help develop stress tolerant and high yielding cultivars or genotypes.
- Partitioning of carbon involves central metabolism, possibly the most well-documented set of pathways; however central metabolism is flexible and context specific, differing in species, tissues and responding to inputs from environment.
- Knowledge gained will assist the corn and soybean breeding communities to select genotypes that use solar and nutrient resources efficiently to maximize yields. Producers will be able to select hybrids or varieties that either are able to tolerate nitrogen- or population stress-environments, or alternatively, select ones that utilize fertilizer more efficiently and that respond to other management practices to obtain even greater yields. The community as a whole will benefit by the crops grown with less fertilizer runoff, thereby decreasing pollution of waterways.
- Our discovery of a rhomboid protease affecting central lipid metabolism in the chloroplast opens up a new paradigm how lipid biosynthesis in chloroplasts and ultimately the assembly of the photosynthetic membrane is regulated in plants. Connecting the FAD4 activity with the redox state of the chloroplast potentially links lipid metabolism with abiotic stress responses protecting photosynthesis under adverse conditions.
- Our research is expected to increase understanding of how the key carboxylating enzyme kinetics and activity control the photosynthetic reduction of CO_2. The outcome from this research will provide a quantitative measure of how individual rate constants combine to determine the overall kinetic properties of Rubisco and the molecular basis of specific kinetic properties of PEPC. It is expected this will enhance our understanding of how the kinetic limitations of these enzymes influence photosynthetic carbon reduction.
- Our determination of reliable biomass production for prickly pear cactus (Opuntia spp.) on arid lands in the USA will lead to more accurate bioenergy production and carbon sequestration estimates from highly productive CAM species in arid environments.
- Our characterization phenotypic diversity within the USDA-ARS germplasm collection of Teff (*Eragrostis tef*) has led to the identification of drought-tolerance accessions of *E. tef* and detailed investigations into the mechanistic basis of their drought tolerance and maintenance of seed and biomass productivity under water-deficit stress conditions.
- This research will provide insights into the genetic mechanisms underpinning photosynthetic rates in soybean and the extent to which they relate to carbon isotope discrimination. It also sheds light on the physiological changes associated with increases in soybean yield over the course of breeding for greater yields. Identified QTL and a better understanding of inadvertent impacts that resulted from breeding for higher yields provide tools for breeders and insights into possible physiological mechanisms that may be targeted for germplasm improvement and increase soybean yield in the years to come.
- Our discovery that a synthetic gene can be used in a model and cover crop species to control Pi acquisition opens the door to Pi remediation strategies to mitigate Pi pollution.
- We have found that flag leaf starch has increased in spring wheat varieties over the last 100 years and that several landraces are sources of much higher flag leaf starch. The long-term impact of this research is identifying ways to increase yield by selecting for increased photosynthetic rates. This in turn would increase productivity and economic return for farmers.
- Abiotic stresses reduce the photosynthetic efficiency of plants. Abiotic stresses such as drought and heat are likely to become severe problems with the predicted global warming. The 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 discovery that evolution in the way in which stomata open, by displacement into epidermal cells, was critical for the evolution of high photosynthetic rates in angiosperms has considerable potential for enhancing our understanding of the anatomical limitations placed on photosynthetic rates in plants.
- Our discovery that a non-native antioxidant improves plant growth by modifying photosynthesis has the potential to modify how plants are cultivated, particularly in indoor environments. A better understanding of how this effect is achieved has the potential to revolutionize precise control of photosynthetic energy diffusion in real time.
- This research is discovering how photosynthesis is regulated to allow processes with widely different time frames and spatial scales to work together. Understanding this regulation is the first step to predicting how photosynthesis might change in the future and what can be done to optimize photosynthesis for human needs.
- This work demonstrates our ability to combine flux analysis with classic approaches in gas exchange to reveal important insights concerning the metabolic drivers of CO_2 release during photosynthesis.

- Our research will help understand the regulation of photosynthesis under abiotic stresses in both green algae and land plants and provide potential targets to improve stress tolerance in photosynthetic organisms.

Publications

1. Agarie S, Umemoto M, Sunagawa H, Anai T, Cushman JC. (2020) An *Agrobacterium*-mediated transformation via organogenesis regeneration of a facultative CAM plant, the common ice plant *Mesembryanthemum crystallinum* L. Plant Production Science 23: 343–349. DOI: 10.1080.1343943x.2020.1730700
2. Aiken, R.M. 2020. Water use and productivity of corn and grain sorghum in long-term crop sequences. Kansas Field Research 2020.
3. AuBuchon-Elder T, Coneva V, Goad DM, Jenkins LM, Allen DK, Kellogg EA. 2020. Sterile spikelets contribute to yield in sorghum and related grasses. Plant Cell 9; doi: 10.1105/tpc.20.00424
4. Bheemanahalli, R., S.M. Impa, I. Krassovskaya, A.R. Vennapusa, K.S. Gill, T. Obata T, S.V.K. Jagadish. 2020. Enhanced N-metabolites, ABA and IAA-conjugate in anthers instigate heat sensitivity in spring wheat. Physiologia Plantarum, 169(4), 501-514.
5. Balfagón, D., A. Gómez-Cadenas, F.B. Fritschi, S. Sengupta, R.K. Azad, R. Mittler, and S.I. Zandalinas. 2019. Jasmonic acid is required for plant acclimation to a combination of high light and heat stress. Plant Physiology 181:1668-1682. DOI: 01.1104/pp.19.00956
6. Bazzer, S.K., A.S. Kaler, J.D. Ray, J.R. Smith, F.B. Fritschi, and L.C. Purcell. 2020. Identification of quantitative trait loci for carbon isotope ratio (α13C) in a recombinant inbred population of soybean. Theoretical and Applied Genetics 133:2141-2155. DOI: 10.1007/s00122-020-03586-0
7. Bernhard, BJ, and Below, FE. 2020. Plant population and row spacing effects on corn: Plant growth, phenology, and grain yield. Agron. J. 112:2456-2465. doi:10.1002/agj2.20245
8. Bernhard, BJ, and Below, FE 2020. Plant population and row spacing effects on corn: Phenotypic traits of positive yield-responsive hybrids. Agron. J. 112:1589-1600. doi:10.1002/agj2.20206
9. Betterle N, Hidalgo Martinez DA, Melis A (2020) Cyanobacterial production of biopharmaceutical and biotherapeutic proteins. Front. Plant Sci. 11:237. DOI: 10.3389/fpls.2020.00237.
10. Boyd, R.D. Cavanagh, A.P., Kubien, D.S., Cousins A.B. (2019) Temperature response of Rubisco kinetics in Arabidopsis thaliana: thermal breakpoints and implications for reaction mechanisms Journal of Exp Botany 70, 231-242
11. Busta L, Shomo ZD, Roston R, Bock N, Pearce M, Flatt P, Terrell C, Jakubowski HV. 2020. Introducing Climate Change into the Biochemistry and Molecular Biology Curriculum. Biochemistry and Molecular Biology Education. doi: 10.1002/bmb.21422
12. Cardoso AA, Brodribb TJ, DaMatta F, McAdam SAM. 2020. Osmotic adjustment and hormonal regulation of stomatal responses to vapor pressure deficit in sunflower. AOBPlants 12: plaa025
13. Cardoso AA, Visel D, Kane CN, Batz TA, Sanches CG, Kaack L, Lamarque LJ, Torrez-Ruiz JM, Corso D, Badel E, Burlet R, Delzon S, Cochard H, Jansen S, McAdam SAM. 2020. Drought-induced lacuna formation in the stem causes hydraulic conductance to decline before xylem embolism in Selaginella. New Phytologist 227: 1804-1817.
14. Casado-García, A., A. Bilbao-Kareaga, A. del-Canto, F.B. Fritschi, J. Heras, M. Lacuesta, J. Miranda-Apocaca, A. Muñoz-Rueda, U. Pérez-López, A. Sanz-Saez, A. Yoldi-Achalandabaso. 2020. LabelStoma: A tool for measuring stomatal density based on the YOLO algorithm. Computers and Electronics in Agriculture 178 (2020) 105751. DOI: 10.1016/j.compag.2020.105751
15. Cohen, I., S.I. Zandalinas, C. Huck, F.B. Fritschi, and R. Mittler. 2020. Meta-analysis of drought and heat stress combination impact on crop yield and yield components. Physiologia Plantarum. DOI: 10.1111/ppl.13203
16. Davidson, J., R.M. Aiken, D. Min and G. Kluitenberg. 2020. Water use and productivity of teff, a dairy quality forage crop. Kansas Field Research 2020.
17. Davis, J., R. Pares, T. Bernstein, S. McDowell, E. Brown, L. Poulsen, J. Stubrich, A. Rosenberg, E. Cahoon, R. Cahoon, M. Palmgren, R. Lopez-Marques, JF. Harper (2020) Two lipid flippases, ALA4 and ALA5, are important for cellular expansion and vegetative growth in Arabidopsis thaliana. Plant Physiol. 182(4) 2111-2125 doi: 10.1104/pp.19.01332
18. Davis, J., R. Pares, M. Palmgren, R. Lopez-Marques, JF. Harper (2020) A potential pathway for flippase-facilitated glucosylceramide catabolism in plants. Plant Signaling and Behavior, 15(10):1783486. doi: 10.1080/15592324.2020.1783486
19. Djanaguiraman, M., S. Narayanan, E. Erdayani, P.V.V. Prasad. 2020. Effect of high temperature stress during anthesis and grain filling periods on photosynthesis, lipids and grain yield in wheat. BMC Plant Biology 20: 268.
20. Gillman, J.D., J.J. Biever, S. Ye, W.G. Spollen, S.A. Givan, Z. Lyu, T. Joshi, J.R. Smith, and F.B. Fritschi. 2019. A seed germination transcriptomic study contrasting two soybean genotypes that differ in terms of their tolerance to the deleterious impacts of elevated temperatures during seed filling. BMC Research Notes 12:522. DOI:10.1186/s13104-019-4559-7
21. Havko N, Das M, McClain A, Kapali G, Sharkey TD, Howe GA (2020) Insect herbivory antagonizes acclimation to elevated temperature in tomato. Proceedings of the National Academy of Sciences USA 117: 2211-2217

22. Herritt, M., and F.B. Fritschi. 2020. Characterization of photosynthetic phenotypes and chloroplast ultrastructural changes of soybean (Glycine max) in response to elevated air temperatures. Frontiers in Plant Science 11:153. DOI: 10.3389fpls.2020.00153.
23. Höhner, H, Pribil, M, Herbstová, M, Lopez, LS, Kunz, H-H, Li, M, Wood, M, Svoboda, V, Puthiyaveetil, S., Leister, D, Kirchhoff H (2020) Plastocyanin is the long-range electron carrier between photosystem II and photosystem I in plants. Proceedings of the National Academy of Sciences USA 117, 15354-15362
24. Hong HTK, Phuong TTB, Wheatley MD, and Cushman JC. (2019). A method for simultaneous isolation of chloroplasts and mitochondria from ice plant. Plant Cell Biotechnology and Molecular Biology. 20: 47–54. DOI: 10.1016/j.pep.2018.11.010
25. Horn PJ, Smith MD, Clark TR, Froehlich JE, Benning C. 2019. PEROXIREDOXIN Q stimulates the activity of the chloroplast 16:1Δ3trans FATTY ACID DESATURASE4. Plant J. 102:718-729. doi: 10.1111/tpj.14657
26. Howell ME, Booth CS, Sikich SM, Helikar T, van Dijk K, Roston RL, Couch BA (2020) Interactive learning modules with 3D printed models improve student understanding of protein structure–function relationships. Biochemistry and Molecular Biology Education.48:356-358. doi: 10.1002/bmb.21362
27. Hu W, Lu Z, Meng F, Li X, Cong R, Ren T, Sharkey TD, Lu J (2020) The reduction in leaf area precedes that in photosynthesis under potassium deficiency: the importance of leaf anatomy. New Phytologist 227: 1749-1763
28. Hultine KR, Cushman JC, Williams DG. (2019) New perspectives on crassulacean acid metabolism. Journal of Experimental Botany. 70: 6489–6493. DOI: 10.1093/jxb/erz465. Cover article.
29. Impa, S.M., A.R. Vennapusa, R. Bheemanahalli, D. Sabela, D. Boyle, H. Walia, S.V.K. Jagadish SVK. 2020. High night temperature induced changes in grain starch metabolism alters starch, protein and lipid accumulation in winter wheat. Plant Cell and Environment, 43(2), 431-447.
30. Jagadish, S.V.K. 2020. Heat stress during flowering in cereals – effects and adaptation strategies. New Phytologist – Tansley Insight. 226(6), 1567-1572.
31. Kaler, A.S., H. Abdel-Haleem, F.B. Fritschi, J.D. Gillman, J.D. Ray, J.R. Smith, and L.C. Purcell. 2020. Genome-wide association mapping of dark green color index (DGCI) using a diverse panel of soybean accessions. Scientific Reports 10:5166. Doi: 10.1038/s41598-020-62034-7
32. Kalra I, Wang X, Cvetkovska M, Jeong J, McHargue W, Zhang R, Hüner N, Yuan J, and Morgan-Kiss R. (2020) Chlamydomonas sp. UWO241 exhibits constitutively high cyclic electron flow and rewired metabolism under high salinity. Plant Physiology. 183: 588-601.
33. Kambhampati S, Aznar-Moreno JA, Hostetler C, Caso T, Bailey SR, Hubbard AH, Durrett TP, Allen DK. 2020. On the inverse correlation of protein and oil: Examining the effects of altered central carbon metabolism on seed composition using soybean fast neutron mutants. Metabolites Journal 10(1): 18; doi:10.3390/metabo10010018
34. Li, M, Mukhopadhyay, R, Svoboda, V, Oung, HMO, Mullendore, DL, Kirchhoff, H (2020) Measuring the dynamic response of the thylakoid architecture in plant leaves by electron microscopy. Plant Direct 2020;00:1-14, DOI: 10.1002/pld3.280
35. Lim SD, Meyer JA, Yim WC, and Cushman JC. (2020) Plant tissue succulence engineering improves water-use efficiency, water-deficit stress attenuation, and salinity tolerance in Arabidopsis. The Plant Journal. 103: 1049–1072. DOI: 10.1111/tpj.14783.
36. Lohaus RH, Zager JJ, Kosma DK, Cushman JC (2019) Characterization of seed oil and fatty acid methyl esters (FAMEs) from wild-type and an ethyl methanesulfonate (EMS) mutant of *Camelina sativa* with reduced seed-coat mucilage. Journal of American Oil Chemists' Society. 97: 157–174. DOI: 10.1002/aocs.12322.
37. Lohaus RH, Neupane D, Mengistu MA, Solomon JKQ, Cushman JC. (2020) Five-year field trial of eight Camelina sativa varieties for biomass to be used for biofuel under irrigated conditions in a semi-arid climate. MDPI Agronomy. 10: 562. DOI: 10.3390/agronomy10040562.
38. Lu Y, Liu L, Roston RL, Soll J, Gao H. (2020) Editorial: Structure and Function of Chloroplasts - Volume II. Frontiers in Plant Science. doi: 10.3389/fpls.2020.620152
39. McClain AM, Sharkey TD (2020) Building a better equation for electron transport estimated from chlorophyll fluorescence: Accounting for non-photosynthetic light absorption. New Phytologist 225: 604-608
40. Maimaitijiang, M., V. Sagan, P. Sidike, S. Hartling, F. Esposito, and F.B. Fritschi. 2020. Soybean yield prediction from UAV using multimodal data fusion and deep learning. Remote Sensing of Environment 237 (2020) 111599. DOI:10.1016/j.rse.2019.111599
41. Meng S, Tan Y, Chang S, Li J, Maleki SJ, Puppala N. (2020) Peanut allergen reduction and functional property improvement by means of enzymatic hydrolysis and transglutaminase crosslinking. Food Chemistry 302:125186.
42. Mitros T, Session A, James BT, Wu GA, Belaffif MB, Clark LV, Shun S, Dong H, Barling A, Holmes JR, Mattick JE, Bredeson JV, Liu S, Farrar K, Głowacka K, Jeżowski S, Barry K, Chae WB, Gifford J, Oladeinde A, Yamada T, Grimwood J, Putnam NH, De Vega J, Barth S, Jin X, Peng J, Yu CY, Heo K, Yoo JH, Ghimire BK, Juvik JA, Klaas M, Hodkinson T, Li L, Donnison IS, Schmutz J, Hudson ME, Sacks EJ, Moose SP, Swaminathan K, Rokhsar DS. 2020. Genome biology of the paleotetraploid perennial biomass crop Miscanthus. Nature Communication (accepted)
43. Moreles-Briones DF, Kadereit G, Tefarikis D, Moore MJ, Smith SA, Brockington SF, Timoneda A, Yim WC, Cushman JC, Yang Y. (2020) Disentangling sources of genes tree discordance in phylogenomic datasets: Testing

ancient hybridizations in Amaranthaceae s.l.. Systematic Biology. DOI: 10.1093/sysbio/syaa066. [Epub ahead of print] PubMed PMID: 32785686.
44. Olatoye MO, Clark LV, Labonte NR, Dong H, Dwiyanti MS, Anzoua KG, Brummer JE, Ghimire BK, Głowacka K, Heo K, Jin X, Nagano H, Peng J, Chang YY, Yoo JH, Zhao H, Long SP, Yamada T, Sacks EJ, Lipka AE. 2020. Training Population Optimization for Genomic Selection in Miscanthus. G3: Genes, Genomes, Genetics 10 (7): 2465-2476. https://doi.org/10.1534/g3.120.401402
45. Ostmeyer, T., R. Bheemanahalli, D. Srikanthan, S. Bean, K.H.S. Peiris, P. Madasamy, R. Perumal, S.V.K. Jagadish. 2020. Quantifying the agronomic performance of new grain sorghum hybrids for enhanced early-stage chilling tolerance. Field Crops Research, 258, 107955.
46. Pandian, B.A., R. Satishraj, M. Djanaguiraman, P.V.V. Prasad, M. Jugulam. 2020. Role of cytochrome P450 enzymes in plant stress response. Antioxidants 9: 454
47. Paudyal, S., J.S. Armstrong, K.L. Giles, W. Hobak, R. Aiken and M.E. Payton. 2020. Differential Responses of Sorghum Genotypes to Sugarcane Aphid Feeding. Planta.
48. Perumal, R., S.S. Tomar, A. Bandara, M. Djanaguiraman, T.T. Tesso, P.V.V. Prasad, H.D. Upadhyaya, C.R. 2020. Variation in stalk rot resistance and physiological traits of sorghum genotypes in the field under high temperature. Journal of General Plant Pathology 86: 350-359.
49. Polle JEW, Calhoun S, McKie-Krisberg Z, Prochnik S, Neofotis P, Yim WC, Hathwaik LT, Jenkins J, Molina H, Bunkenborg J, Grigoriev I, Barry K, Schmutz J, Jin E, Cushman JC, Magnusson JR. (2020) Genomic adaptations of the green alga Dunaliella salina to life under high salinity. Algal Research. 50: 101990. DOI: 10.1016/j.algal.2020.101990
50. Sadok, W., S..V.K. Jagadish. 2020. The hidden costs of nighttime warming on yields. Trends in Plant Science, 25(7), 644-651.
51. Santiago JP, Ward JM, Sharkey TD (2020) Phaseolus vulgaris SUT1.1 is a high affinity sucrose-proton co-transporter. Plant Direct 4: e00260
52. Šebela, D., B. Bergkamp, S.M. Impa, A.K. Fritz, S.V.K. Jagadish. 2020. Impact of post-flowering heat stress on spike and flag leaf senescence in wheat tracked through optical signals. Agronomy Journal, 112(5), 3993-4006
53. Sheehan H, Feng, T, Walker-Hale N, Lopez-Nieves S, Pucker B, Guo R, Yim WC, Timoneda A, Zhao L, Tiley H, Copetti D, Sanderson MJ, Cushman JC, Moore MJ, Smith SA, Brockington SF (2019) Evolution of L-DOPA 4,5-dioxygenase activity allows for recurrent specialisation to betalain pigmentation in Caryophyllales. New Phytologist. 227: 914–929. DOI: 10.1111/nph.16089.
54. Soba, D., T. Shu, G.B. Runion, S.A. Prior, F.B. Fritschi, I. Aranjuelo, and A. Sanz-Saez. 2020. Effects of elevated [CO2] on photosynthesis and seed yield parameters in two soybean genotypes with contrasting water use efficiency. Environmental and Experimental Botany 178 (2020) 104154. DOI: 10.1016/jenvexpbot.2020.104154
55. Sharkey TD (2020) Emerging research in plant photosynthesis. Emerging Topics in Life Sciences 4: 137-150
56. Sharkey TD, Preiser AL, Weraduwage SM, Gog L (2020) Source of 12C in Calvin Benson cycle intermediates and isoprene emitted from plant leaves fed with 13CO2. Biochemical Journal 477: 3237-3252
57. Sunoj, V.S.J., P.V.V. Prasad, I.A. Ciampitti, H.E. Maswada. 2020. Narrowing diurnal temperature amplitude alters carbon tradeoff and reduces growth in C4 crop sorghum. Frontiers in Plant Sciences 11: 1262.
58. Tietz, S, Leuenberger, M, Höhner, R, Olson, AH, Fleming, GR, Kirchhoff, H (2020) A proteoliposome-based system reveals how lipids control photosynthetic light harvesting (2020) Journal of Biological Chemistry 295, 1857-1866
59. (Article was picked as Editor's choice in Science)
60. Tran DQ, Konishi A, Cushman JC, Morokuma M, Toyota M, Agarie S. (2019) Ion accumulation and expression of ion homeostasis-related genes associated with halophytism, NaCl-promoted growth in a halophyte, Mesembryanthemum crystallinum L. 9: 91–102. Protein Production Science. DOI: 10.1080/1343943X.2019.1647788.
61. Vogel, AM and Below, FE. 2020. Residue and agronomic management to reduce the continuous corn yield penalty. Agronomy 9:567. doi:10.3390/567
62. Walker, B.J., et al., Flexibility in the Energy Balancing Network of Photosynthesis Enables Safe Operation under Changing Environmental Conditions. Plants, 2020. 9(3): p. 301
63. Weigand, C, JF. Harper, Decapitation Crosses to Test Pollen Fertility Mutations for Defects in Stigma-style Penetration (2020) In: Pollen and Pollen Tube Biology: Methods and Protocols, Anja Geitmann (editor), MIMB, 2160:29-40. doi: 10.1007/978-1-0716-0672-8_3
64. Westbrook AS, McAdam SAM. 2020. Atavistic stomatal responses to blue light in Marsileaceae. Plant Physiology doi: 10.1104/pp.02.00967
65. Westbrook AS, McAdam SAM. 2020. Stomatal density and mechanics are critical for high productivity: insights from amphibious ferns. New Phytologist doi: 10.1111/nph.16850
66. Yang X, Cushman JC, Borland AM, Liu Q. (2020) Systems biology and synthetic biology in relation to drought tolerance and avoidance in plants. Frontiers in Plant Science. 11: 394. DOI: 10.3389/fpls.2020.00394.
67. Zandalinas, S.I., I. Cohen, F.B. Fritschi, and R. Mittler. 2020. Coordinated systemic stomatal responses in a crop plant. Plant Physiology 183:1428-1431. DOI: 10.1104.20.00511
68. Zandalinas, S.I., F.B. Fritschi, and R. Mittler. 2020. Signal transduction networks during stress combination. Journal of Experimental Botany. 71:1734-1741. DOI: 10.1093/jxb/erz486

69. Zhang J, Hu R, Sreedasyam A, Garcia TM, Lipzen A, Wang M, Yerramsetty P, Liu D, Ng V, Schmutz J, Cushman JC, Borland AM, Pasha A, Provart NJ, Chen J-G, Muchero W, Tuskan GA, Yang X. (2020) Light-responsive expression atlas reveals the effects of light quality and intensity in Kalanchoë fedtschenkoi, a plant with crassulacean acid metabolism. GigaScience. 9: 1-17. DOI: 10.1093/gigascience.gia018
70. Zhou XR, Bhandari S, Johnson B, Kotapati HK, Allen DK, Vanhercke T, Bates PD. 2019. Reorganization of acyl flux through the lipid metabolic network in oil-accumulating tobacco leaves. Plant Physiology 182(2):739-755