Annual Report of NC1200: Regulation of Photosynthetic Processes
Period the Report Covers: 10/2013 to 09/2014
Administrative Advisor: C. Benning and S. Pueppke (Michigan State University)
Date of Annual Report: 4/27/2015

Cooperating Institutions and Principal Leaders

CA- AES T. Melis
FL- AES K. Koch
IL- AES/ARS F. Below, L. Gentry, S. Huber
IA- AES S. Rodermel, M. Spalding
KS- AES R. Aiken, V. Prasad
LA- AES J. Moroney
MI- AES C. Benning, D. Kramer, W. Loescher, T. Sharkey
MN- AES J. Erwin
MS- AES J. Li
MO- AES F. Fritschi
MT- AES M. Gioux
NE- AES R. Spreitzer, J. Stone, D. Weeks
NV- AES J. Cushman, J. Harper
OH- AES J-C Jang
VA- AES G. Gillaspy
WA- AES G. Edwards, T. Okita

Summary of Minutes of the Annual Meeting
Annual Meeting Date and Location
November 14-15, 2014; Kansas AES host; Embassy Suites, Kansas City, MO

Attending Members

Dr. Tasios Melis	California AES
Dr. Fred Below	Illinois AES
Dr. Steve Huber	Illinois ARS
Dr. Rob Aiken	Kansas AES
Dr. Vara Prasad	Kansas AES
Dr. Christoph Benning	Michigan AES
Dr. Tom Sharkey	Michigan AES
Dr. Jiaxu Li	Mississippi AES
Dr. Felix Fritschi	Missouri AES
Dr. Julie Stone	Nebraska AES
Dr. John Cushman	Nevada AES

Attending Guests, interested in NC-1200 Membership*

Dr. Ruth Welti	Kansas AES
Dr. Asaph Cousins*	Washington AES
Dr. Rebecca Roston*	Nebraska AES

Agenda of Meeting:

Friday, Nov. 14, 2014 Evening social in Atrium of Embassy Suites Hotel

Saturday, Nov. 15, 2014 Breakfast in Atrium of Embassy Suites Hotel

8:30 Announcements and introduction, Rob Aiken; 8:45 Welcome by Kansas AES Associate Director Ernie Minton; 9:00 John Cushman, Nevada AES; 9:30 Asaph Cousins, Washington AES; 10:00 Break; 10:15 Tasios Melis, California AES; 10:45 Julie Stone, Nebraska AES; 11:15 Rebecca Roston, Nebraska AES

11:45 Business Meeting I; 12:00 Lunch in Atrium of Embassy Suites Hotel

1:00 Steve Huber, Illinois USDA-ARS; 1:30 Christoph Benning, Michigan AES; 2:00Tom Sharkey, Michigan AES; 2:30 Fred Below, Illinois AES; 3:00 Break; 3:15 Rob Aiken, Kansas AES; 3:45 Vara Prasad, Kansas AES; 4:15 Jiaxu Li, Mississippi AES; 4:45 Felix Fritschi, Missouri AES; 5:15 Business Meeting II.

Minutes of Business Meeting I:

1. The renewal proposal was approved effective October 1, 2012 – September 30, 2017; a mid-period review is underway, involving Christoph Benning as Administrator of the NC1200. Participants encouraged to think about: i) Group history; and ii) Can we identify, highlight and strengthen collaborations.
2. Christoph Benning indicated his original involvement with the group was prompted by substantial support from the AES. As Adminstrator, he (with a writer) wrote up article last year highlighting the group and contributions; this resulted in good publicity. The group brings together people from diverse backgrounds.
3. Discussion continued regarding support from respective AES. One member indicated AESs are required to spend some money to fund these Regional Research groups. Another stated that AES funds also go into retention and start-up packages. One member indicated AES doesn't fund his project or salary. Another reported that no money to the lab, but part salary from AES; also benefit from less teaching.
4. Members considered application of prospective members: Asaph Cousins and Rebeca Roston. The question: do both applicants have AES partial appointments? Yes. Collaborative interactions were addressed, in support of applicants. Motion to approve membership carried, unanimous by voice vote.
5. The group identified the need for a volunteer to host the 2016 meeting; also discussed development of the application for renewal.

Business Meeting II

6. From prior meeting notes and discussion, active collaboration among members addresses investigation of TALEN's (Weeks, Spalding, Huber), soybean stress response (Prasad and Fritschi), Isoprene biosynthesis (Sharkey and Fritschi), photosynthate partitioning (Benning, Kramer and Sharkey), stress responses (Kramer, Sharkey, Fritschi),

Rubisco Activase (Huber, Spalding and Salvucci; Spreitzer and Salvucci), C assimilation (Kramer and Cousins), lipids (Stone and Roston.

7. From members present, reconstruction of previous meeting hosts:

2001 NC 142 NC AES
2002 NC 1-142OR AES
2003 NC 1-142
2004 NC 1-142SC AES
2005 NC 1-142FL AES
2006 NC 1-142KY AES
2007 NC 1168 NE AES
2008 NC 1168 AZ AES
2009 NC 1168 OH AES
2010 NC 1168 VT AES
2011 NC 1168 MS AES
2012 NC 1200 NV AES
2013 NC 1200 NE AES
2014 NC 1200 KS AES

8. Host of the 2015 meeting was discussed; WA, MS, MT, MO, IA, CA are possibilities as haven't hosted previous meetings. Felix Fritschi (MO AES) offered to host 2015 meetings either in Columbia or St. Louis. Motion to approve (Christoph Benning), seconded by Tom Sharkey, approved by unanimous vote.
9. Host of 2016 meeting to be resolve at 2015 meeting. General discussion continued on AES support for participation and effort involved in writing the renewal. Members can decide if they want to renew membership and if so, participate in renewal writing. Members are advised to check with their AES to see if there is incentive for participation in the group.

5:40 PM, meeting adjourned. Bus leaves at 7:15PM for a 7:30PM dinner reservation (Swagat).

Accomplishments:

Summary of Accomplishments Presented by Meeting Attendees and in submitted Reports

Objective 1. Plastid Function and Intracellular Communication

Stone (Nebraska AES)

1. Using molecular genetics approaches, we have identified knockout mutants for some of the *A. thaliana* DJ-1 homologs. At least one gene is essential for viability. Two *AtDJ1C* gene T-DNA insertion mutant alleles confer an albino, seedling-lethal phenotype.
2. Wild-type DJ1C protein is targeted to chloroplasts and mutant, albino plant tissues (cotyledons) have severely disrupted chloroplast ultrastructure indicating that the protein normally functions in chloroplast biogenesis.

3. The chloroplast biogenesis defect associated with *dj1c* null mutations is effectively complemented with a functional epitope-tagged version of the protein (pDJ1C:gDJ1C:GFP).
4. AtDJ1C partial loss-of-function (RNAi) lines with varying levels of *DJ1C* expression (and varying levels of chloroplast dysfunction) have been generated, and are now under analyses.

Objective 2. Photosynthetic Capture and Photorespiratory Release of CO2

Melis (California AES)

1. Successfully applied the TLA concept to cyanobacteria mass cultures.

Moroney (Louisiana AES)

1. Identification of new insertional mutants of *Chlamydomonas reinhardtii* unable to grow normally on low concentrations of CO_2.
2. Successfully expressed the *Chlamydomonas reinhardtii* CCM transport protein NAR1.2 in higher plants.
3. Constructed knock-out strains of *Arabidopsis thaliana* missing one or more carbonic anhydrase proteins. Some of these plants cannot grow normally on certain light and CO_2 growth regimes.

Spreitzer (Nebraska AES)

1. In collaboration with M. E. Salvucci (Arizona ARS), research demonstrated that Rubisco small subunits do not influence the species specificity of interactions between Rubisco and Rubisco activase.
2. By employing directed mutagenesis and chloroplast transformation, elucidated the functional and structural significance of the 34 large-subunit residues that differ between algal and plant Rubisco enzymes.
3. By employing directed mutagenesis and chloroplast transformation, discovered that posttranslational modifications (hydroxy-Pro and methyl-Cys) in the large subunit of *Chlamydomonas* Rubisco are more important for catalysis than for holoenzyme assembly.
4. Succeeded in expressing functional *Arabidopsis* Rubisco in *Chlamydomonas* by changing only 12 large-subunit residues.
5. Succeeded in expressing functional *Rhodospirillum rubrum* Rubisco from the nucleus in a *Chlamydomonas* strain that lacks the genes for both the *Chlamydomonas* large and small subunits.

Weeks (Nebraska AES)

1. Demonstration of highly efficient gene editing in higher plants nd stable inheritance of the edited genes.
2. Creations of CRISPR/Cas9/sgRNA systems that allow for simultaneous knockout of multiple genes and/or the creation of very large (>250 kbp) chromosomal deletions.

3. Successful demonstration of transient expression of the CRISPR/Cas9 system in the model algal cell, *Chlamydomonas reinhardtii*.

Edwards and Okita (Washington AES)

1. The cell specific expression of biochemistry to form C_4 photosynthesis was found to occur very early during leaf development in C_4 species in the eudicot family Cleomaceae prior to structural differentiation. In *Cleome angustifolia* and *C. gynandra* development of the C_4 system occurs similarly, irrespective of these species having very different types of Kranz anatomy, different ontogenetic origins of bundle sheath and mesophyll, and independent evolutionary origins of C_4 photosynthesis.
2. Besides C_4 plants having high levels of phosphoenolpyruvate carboxylase (PEPC), the C_4 type enzyme has altered kinetic properties. There is evidence for this in C_4 monocots, with positive selection occurring for certain amino acid residues. In a study of PEPC among species in the eudicot family Chenopodiaceae, we find differences. There is divergence in amino acid substitutions indicating the same substitutions found in monocots are not required in PEPC for function of C_4 photosynthesis.
3. A unique structural form of C_4 photosynthesis occurs in some species in family Chenopodiaceae, not by the dual-cell Kranz system, but rather within individual photosynthetic cells which contain two types of chloroplasts. One chloroplast type has pyruvate,Pi dikinase for function in the carboxylation phase of the C_4 cycle, the other has Rubisco which functions in the C_3 cycle, but how this differentiation occurs is unknown. In the single cell C_4 species, *Bienertia sinuspersici*, transient expression analysis using GFP fusion constructs containing various lengths of Rubisco small subunit (RbcS) gene and the transit peptide of PPDK revealed that their import was not specific to either chloroplast type, indicating lack of selectivity in import. Post-transcription regulation is proposed to control selective expression of Rubisco. A post-transcriptional regulator factor RLSB (Rubisco large subunit mRNA binding factor) was found to be selectively localized in the Rubisco containing chloroplast. RLSB, which also is selectively localized in Rubisco containing chloroplasts in Kranz type C_4 species, is proposed to mediate selective accumulation of Rubisco in chloroplasts in C_4 systems, including single cell C_4 species.
4. In the single-cell C_4 *Bienertia* species, decarboxylation of C_4 acids in the C_4 cycle occurs via NAD-malic enzyme in mitochondria which are surrounded by Rubisco containing chloroplasts. There is interest in introducing C_4 traits into C_3 crops, e.g. rice. Rice mesophyll cells have chloroplasts in lobes at the cell periphery and mitochondria located internally. In a recent review we discuss features of terrestrial and aquatic plants having single cell C_4 photosynthesis. Incorporation of an NAD-ME type C_4 cycle in rice mesophyll cells was proposed, in the interest of providing supplemental CO_2 to Rubisco to increase photosynthesis and reduce photorespiration.

Objective 3. Mechanisms Regulating Photosynthate Partitioning

Koch (Florida AES)

1. We demonstrated a strigolactone-based alteration in C-partitioning to maize kernels. The primary shift observed in strigolactone-deficient mutants was related to changes in the underlying and adjacent maternal tissues. We also began genetic work to test whether the observed yield penalty in this material could be ameliorated by hybrid vigor or other means. This mutant offers considerable potential for resistance to *Striga*, the parasitic African weed that devastates maize yields there.
2. We identified a potential regulatory contribution by G4 DNA motifs to C-responsive genes. These motifs can form complex tertiary structures *in vivo* and alter both transcription and translation. We found that G4 motif hotspots were enriched in key regulatory genes for hypoxia, oxidative stress, and energy status pathways. They were also statistically more abundant in genes for enzymes of glycolysis, sugar degradation, and inositol metabolism. The G4 motifs tested *in vitro* formed the predicted G4 DNA structures.
3. A total of 1,728 new, tagged-mutant maize lines were released this year (2014). These carry >15,000 mapped insertions in a background that provides uniform, wildtype controls (W22 inbred). The Uniform Mu Maize Resource now includes multiple insertions in ~40% of maize genes. Seeds are sequence-indexed and available via MaizeGDB.org and the Maize Genetics Cooperative Stock Center.
4. We developed a protocol for effective forward genetics identification of genotypes from phenotypes in the UniformMu population of transposon-tagged maize mutants. Mu-seq protocols were adapted for use with multiplexed samples from mutants in gridded arrays. Sites of Mu insertions are mapped by anchoring reads to transposon edges (terminal inverted repeats), then sequencing and mapping adjacent host DNA.

Huber (Illinois AES/ARS)

1. Identified phosphorylation of Rubisco activase (RCA) at the Thr-78 site that may function in concert with redox regulation to modulate Rubisco activation state in response to light-dark transitions (collaboration with Marty Spalding and Mike Salvucci).
2. Determined that Ser-Thr substitutions at phosphosites can be an interesting approach to alter regulatory mechanisms. The specific example was the BRI1 receptor kinase where Ser891 (a known phosphosite) was substituted with a Thr; the S891T recombinant protein autophosphorylated more slowly than the wild type protein during expression in *E. coli* and as a result, activation of peptide kinase activity (measured in vitro) was delayed as was trans-phosphorylation of bacterial proteins *in situ*.
3. The BAK1 receptor kinase is spontaneously glutathionylated *in vitro* by GSSG or GSH plus H_2O_2. Glutathionylation can be catalyzed by the glutaredoxin, AtGRXC2, and inhibits BAK1 kinase activity towards a synthetic peptide substrate. Results establish the potential for redox control of BAK1 and demonstrate a novel activity of a glutaredoxin from plants.

Benning (Michigan AES)

1. Characterized the CHT7 transcription factor complex in Chlamydomonas and showed that it acts as a repressor of transcriptional programs of nutrient-dependent quiescence in Chlamydomonas.
2. Conducted high-throughput screen for lipid mutants in Nannochloropsis.
3. Identified a role of Arabidopsis PGP1-like lipase during senescence and characterized the activity of the PDG1-like enzymes in vitro.
4. Completed a model and biochemical analysis of SFR2, which is involved in oligogalactolipid biosynthesis and indirectly in leaf triacylglycerol accumulation during freeze stress in Arabidopsis.
5. Characterized a TGD2 lipid trafficking mutant in Chlamydomonas, which shows an increase in triacylglycerol accumulation, but accelerated cell death.
6. Designed and introduced constructs expressing algal lipid genes into Brachypodium.
7. Investigated the expression of WRI1 in Brachypodium and its effect on triacylglycerol accumulation and premature cell death.

Giroux (Montana AES)

1. Created transgenic rice with overexpression of leaf and/or seed starch biosynthetic rates.
2. Completed initial yield trial with data to be submitted for publication in 2015.
3. Completed initial photosynthetic rate studies comparing the impact of increased leaf and/or seed starch upon whole plant growth.

Cushman (Nevada AES)

1. Microalgae can serve as useful feedstocks for biofuel production as they can be grown with fresh, brackish, or salt water and their lipid and starch contents can be manipulated to create customized feedstocks for different classes of biofuels. Various selection methods, based upon flow cytometry, fluorescence-activated cell sorting, and buoyant density centrifugation, have been used successfully to select and isolate algal strains with altered feedstock characteristics such as greater starch or lipid accumulation (Hathwaik and Cushman, 2014). Continuous buoyant density gradient centrifugation (CBDGC) was used to perform reiterative, transgressive selection to isolate wildtype and ethyl methanesulfate-mutagenized *Dunaliella salina* cells with enhanced lipid and starch production. Sixty rounds of transgressive selection resulted in the isolation of cell populations with significantly lower or higher buoyant densities. Lipid content in the low-density populations was enhanced by 1.2- to 2.9-fold in wildtype cells and 1.3- to 2.3-fold in mutagenized cells as measured by Nile Red dye staining, but the lipid content differences were not significant when quantified by liquid chromatography-tandem mass spectroscopy possibly due to the composition of the lipid pools measured by these contrasting techniques. In contrast, starch content in the high-density populations was increased by 2-fold in wild type cells and 1.4- to 1.6-fold in mutagenized cells, respectively. The observed alterations in lipid and starch contents appeared to be stable after more than 70 weeks (392 cell generations). CBDGC-based selection provides a

useful and accessible technological alternative to genetic engineering approaches for the customization of microbial biofuel feedstocks (Hathwaik et al., 2014a).

Jang (Ohio AES); also Objective 4

1. Demonstrated that Arabidopsis TZF1 binds AU-rich RNA elements via a unique RNA binding domain.
2. AtTZF1 can target specific mRNA for degradation.
3. Identification of AtTZF1 and five interacting partners.
4. Genomic characterization of plant RR-TZF proteins.

Gillaspy (Virginia AES)

1. Delineated the spatial expression profiles, subcellular location, and impacts on biomass of variants of the SnRK1.1 and SnRK1.2 genes.
2. Characterized how plants synthesize potential low energy signaling molecules called inositol pyrophosphates and developed methods to measure this molecule in plants.
3. Characterized the function of a gene (called P80) involved in regulating low energy responses in plants, and used viral-induced gene silencing to suppress expression of this gene in cotton.

Edwards and Okita (Washington AES)

1. In rice, the endosperm cytosolic AGPase isoform is encoded by the *OsAGPS2b* and *OsAGPL2* genes, which code for the small (S2b) and large (L2) subunits of the heterotetrameric enzyme, respectively. Several missense and null *OsAGPL2* mutants were identified, which showed comparable seed weights and starch content. Kinetic analysis showed that the catalytic and regulatory properties of recombinant heterotetrameric enzymes containing the missense L2 mutations and S2b homotetramer enzyme were significantly impaired. Overall, these results showed that the L2 subunit is essential for the enzyme's catalytic and allosteric regulatory properties.
2. The catalytic activity of the major rice endosperm AGPase is controlled by redox potential. Site-directed mutagenesis studies showed that, unlike the leaf and tuber enzymes whose catalytic activity is altered by the interchain disulfide bond formation between the two small subunits, the catalytic activity of the rice endosperm cytoplasmic enzyme was regulated by the redox status of the large subunits. Site directed mutagenesis of the conserved cysteine residues at the N-terminal of the large subunit showed that C47 and C58, but not C12, are essential for proper redox-response of the enzyme. Overall, the catalytic activity of the rice endosperm cytoplasmic AGPase is regulated by allosterism and redox potential.
3. RNAseq of transgenic rice lines over-expressing a bacterial AGPase showed a pronounced elevation of transcripts for a starch binding domain protein while RNAs coding for other known starch biosynthetic enzymes were up-regulated or down-regulated depending on the RNA species. Immunoblot analysis using available antibodies showed that these changes in gene expression at the RNA level were extended to the protein level as well.

4. A unique structural feature of Pho1 that is not present in non-plant eucaryotic phosphorylases is that the higher plant enzyme contains an extra 80 amino acid peptide located near the middle of the primary sequence. Kinetic analysis of the wildtype Pho1 and Pho1 lacking the L80 peptide (Pho1-Δ80) showed no significant differences in catalytic properties suggesting a potential regulatory role for L80 peptide. To elucidate the potential role of the L80 peptide, Pho1 null rice plants transformed with wildtype Pho1 and Pho1-Δ80 were generated and are presently under study.
5. RNAs that code for the rice prolamine storage protein are transported and localized on the ER membrane that delimit the prolamine protein body. Previous studies have shown that a complex collection of RNA binding proteins (RBPs) specifically recognized the RNA-cis-determinants that govern RNA localization. By using a variety of techniques, we demonstrated that five of these RBPs assemble in at least three multi-protein complexes. The complexity of RBPs and their organization into different multi-protein complexes is consistent with the multiple steps required for RNA localization, a process that initiates in the nucleus and terminates by translation at the ER destination site.

Objective 4. Developmental and Environmental Limitations to Photosynthesis

Below and Gentry (Illinois AES)

1. The middle region of the soybean canopy accounts for 60% of the yield potential.
2. Fertilizer and foliar protection treatments can increase soybean yield by 5% due to increases in the seed filling duration and seed weight.
3. Maize hybrids differ in their tolerance of above-average plant densities.
4. A full complement of beneficial crop management inputs is necessary for achieving the greatest maize yields, primarily through increases in kernel number.
5. Preliminary results show that annual ryegrass can be used in combination with minimal nitrogen inputs to grow tropical maize in a sustainable system.
6. Dual- purpose tropical maize hybrid silage has nutritional quality comparable to conventional corn, has high- palatability, and may be grown with less fertilizer.

Aiken and Prasad (Kansas AES)

1. Screened 250 genotypes of spring wheat association mapping (AM) panel to characterize genetic variability for root traits and determine whether root traits are related to above ground traits (Narayanan and Prasad, 2014). There was significant genetic variation for several root traits and we identified few genotypes which has positive root traits. Shoot dry weight had positive relationships ($r > 0.50$) with rooting depth, but there was no correlation of plant height with most root traits. The genetic variability identified in this research for root traits offers useful information for wheat improvement programs for choosing genotype with contrasting root characteristics (Narayanan and Prasad, 2014).
2. Cultivated Wheat Collection (CWC) of 297 genotypes developed at Washington State University was evaluated for root characteristics under controlled environment

(Narayanan et al. 2014). Wheat genotypes collected from Australia, Mediterranean, and west Asia had greater rooting depth than those from south Asia, Latin America, Mexico, and Canada. Soft wheat had greater rooting depth than hard wheat in the CWC germplasm. The genetic variability identified in this research for root traits can be exploited to improve drought tolerance and/or resource capture in wheat (Narayanan et al. 2014).

3. Response of sorghum genotypes (hybrids and inbred lines) to nitrogen fertilizer and relationship between physiological and yield traits was quantified in field conditions (Mahama et al., 2014). Overall hybrids were superior to the inbred lines for grain yield and total aboveground biomass. There was no strong relationship between chlorophyll index or chlorophyll fluorescence and grain yield in this set of genotypes, but a strong relationship was found between seed number and grain yield, and total aboveground biomass and grain yield.
4. The response of eight sorghum genotypes to high temperature (HT) was quantified under controlled environment conditions (Djanaguiraman et al., 2014). HT stress increased leaf and pollen oxidative stage. Sorghum genotypes differed for percentage seed-set under high temperature stress. Genotypes with high Tmax and pollen germination had improved percentage of seed-set under high temperature stress. This research suggest that HT stress tolerant genotypes can be identified by quantifying oxidative damage in pollen grains and by measuring pollen germination, seed-set percentage and grain yield at HT.
5. We partitioned leaf hydraulic resistance of six genotypes of *Sorghum bicolor* L. (Moench) into leaf specific hydraulic resistance within the large longitudinal veins (*r** LV) and outside the large veins (*r** OLV), and correlated these resistances with the response of stomatal conductance (*g*s) and photosynthesis (*A*) to drought (Ocheltree et al., 2014. Under well-watered conditions, *g*s was tightly correlated with *r** OLV (r^2 = 0.95), but as soil moisture decreased, *g*s was more closely correlated with *r** LV (r2 = 0.97). These results suggest that *r** OLV limits maximum rates of gas exchange, but the ability to efficiently move water long distances (low *r** LV) becomes more important for the maintenance of cell turgor and gas exchange as soil moisture declines. Hydraulic resistance through the leaf was negatively correlated with evapotranspiration ($P < 0.001$) resulting in more conservative water use in genotypes with large leaf resistance. These results illustrate the functional significance of leaf resistance partitioning to declining soil moisture in a broadly-adapted cereal species.
6. We found significant genetic variations for grain carbon isotope (13C) discrimination (CID) among wheat breeding lines and a significant and positive correlation between grain CID and yield across three of four field trials conducted under various environmental conditions. These results indicate that grain CID could be a useful selection criterion for grain yield improvement under moderate drought stress, especially post-anthesis drought, in temperate semi-arid regions. The positive relationships of CID and test weight suggests further investigation of CID as an indicator of enhanced translocation capacity.

Li (Mississippi AES)

1. Ten differential phosphoproteins in response to abscisic acid treatments have been identified as enzymes involved in carbohydrate and amino acid metabolism in Arabidopsis.
2. In collaboration with Dr. Vincent Klink at Mississippi State University, over-expression and under-expression (RNAi) lines of abscisic acid-activated protein kinases have been generated in soybean.

Cushman (Nevada AES)

1. Engineering crassulacean acid metabolism (CAM) into C_3 crops to improve water-use efficiency**.** Global climate change threatens the sustainability of agriculture and agroforestry worldwide through increased heat, drought, surface evaporation, and associated soil drying. Exposure of crops and forests to warmer and drier environments will increase leaf:air water vapor-pressure deficits (VPD), and result in increased drought susceptibility and reduced productivity, particularly in arid regions, but also in tropical regions with seasonal dry periods. One approach to sustaining plant productivity is to improve water-use efficiency (WUE) by engineering crassulacean acid metabolism (CAM) into C_3 crops. Current research efforts focus on the comprehensive systems-level understanding of the enzymatic and regulatory pathways underpinning this temporal CO_2 pump. As CAM arose through multiple independent evolutionary origins, comparative transcriptomics and genomics of taxonomically diverse CAM species are being used to define the genetic 'parts list' required to operate the core CAM functional modules of nocturnal carboxylation, daytime decarboxylation, and inverse stomatal regulation. Bioengineered CAM offers the potential to sustain plant productivity for food, fibre, and biofuel production in hotter and drier climates (Borland et al., 2014a; DePaoli et al., 2014). Fast-growing, short-rotation forestry (SRF) bioenergy crops such, as poplar (*Populus* spp.) and willow (*Salix* spp.), are particularly susceptible to hydraulic failure following drought stress due to their isohydric nature and relatively high stomatal conductance. CAM improves WUE by shifting stomatal opening and primary CO_2 uptake and fixation to the nighttime when leaf:air VPD is low. CAM members of the tree genus *Clusia* exemplify the compatibility of CAM performance within tree species and highlight CAM as a mechanism to conserve water and maintain carbon uptake during drought conditions. The introduction of bioengineered CAM into SRF bioenergy trees is a potentially viable path to sustaining agroforestry production systems in the face of a globally changing climate (Borland et al., 2014b).
2. Characterization of evolutionary progression of the phospho*enol*pyruvate carboxylase (PEPC) gene family in neotropical orchids. Phospho*enol*pyruvate carboxylase (PEPC) catalyzes the initial fixation of atmospheric CO_2 into oxaloacetate and subsequently malate. Nocturnal accumulation of malic acid within the vacuole of photosynthetic cells is a typical feature of plants that perform crassulacean acid metabolism (CAM). PEPC is a ubiquitous plant enzyme encoded by a small gene family and each member encodes an isoform with specialized function. CAM-specific *Ppc* gene isoforms likely evolved from ancestral non-photosynthetic isoforms by gene duplication events and subsequent

acquisition of transcriptional control elements that mediate increased leaf- or photosynthetic tissue-specific mRNA expression. To understand the patterns of functional diversification related to the expression of CAM, *Ppc* gene families and photosynthetic patterns were characterized in 11 closely related orchid species from the Oncidiinae with a range of photosynthetic pathways from C_3 photosynthesis to weak CAM, and strong CAM. Phylogenetic analysis revealed the existence of two main *Ppc* lineages in flowering plants, two main lineages within the eudicots, and three *Ppc* lineages within the Orchidaceae. Our results indicate that *Ppc* gene family expansion within the Orchidaceae is likely the result of gene duplication events followed by adaptive sequence divergence. Futhermore, theses results suggest that CAM-associated *Ppc* isoforms in the Orchidaceae probably evolved from several independent origins (Silvera et al., 2014).

3. Characterization of the biomass of *Agave* and *Opuntia* as potential biofuel feedstocks. Sustainable production of lignocellulosic biofuels requires a sufficient supply of biomass feedstocks. *Agave* and *Opuntia* represent highly water-use efficient bioenergy crops that are suitable for expanding feedstock production into semi-arid, abandoned, or degraded agricultural lands. These feedstocks have garnered interest as dedicated biofuel feedstocks because of their high water- and fertilizer-use efficiency and because they do not compete for the use of agricultural lands otherwise used for the production of food crops or conventional biofuel feedstocks. To better understand the potential of these feedstocks, the biomass composition of *Agave tequilana* and *Opuntia ficus-indica* was analyzed. Previous extraction procedures and analytical methods have led to variable estimates of the chemical compositions of the biomass of these species. Therefore, National Renewable Energy Laboratory (NREL) standard methods were used and the results compared to earlier studies. *A. tequilana* showed higher percentages of water-soluble constituents, structural carbohydrates, cellulose, hemicellulose, and lignin than did *O. ficus-indica*. In contrast, *O. ficus-indica* had higher protein, water, and ash content than *A. tequilana*. Both species had lower lignin contents and thus lower heating values, but higher water and ash contents than most woody biomass feedstocks. The high water content of these species (85–94%) could prove advantageous for biomass deconstruction and aqueous phase catalytic conversion processes as less input water would be needed. Lastly, solid-state NMR analysis revealed that both *A. tequilana* and *O. ficus-indica* had high amorphous and para-crystalline cellulose contents (>80%) and low crystalline cellulose contents, indicating that these biomass feedstocks would be far less recalcitrant to deconstruction than are traditional lignocellulosic biomass feedstocks (Yang et al., 2014).

Impact Statements:

Melis (California AES)

Solar energy conversion efficiencies and productivities in mass cultures of green microalgae and cyanobacteria under bright sunlight are 25% or less than the expected theoretical maximal performance. Minimizing, or truncating, the chlorophyll antenna size of the photosystems can

improve photosynthetic solar energy conversion efficiency and productivity under these conditions by up to 4-fold. This concept (the TLA concept) may find application in the commercial exploitation of microalgae and plants for the generation of biomass and valuable bioproducts.

Koch (Florida AES)

A contribution by strigolactone to C-partitioning into maize kernels has been demonstrated. Effects are mediated by maternal structures beneath and adjacent to kernels.

A maize mutant deficient in strigolactone biosynthesis continues to hold promise as a source of maize resistance to *Striga* (a deadly parasitic weed that reduces yields to near zero in much of Africa and elsewhere in the developing world).

An additional contribution to regulatory mechanisms of C-responsive genes is implicated for G4 quadruplex structures that form tertiary folds in DNA. These kinks can alter transcription and are especially abundant in genes responsive to C-starvation and cellular energy state.

Knock-out and knock-down mutants are now available for ~40% of maize genes. These mutants provide invaluable tools for determining gene function. Each is transposon-tagged, mapped, and sequence-indexed as part of the UniformMu Public Resource (searchable by sequence at MaizeGDB.org). Seeds are now available for all mutants, free of charge, through MaizeGDB.org, and the Maize Genetics Cooperative Stock Center. Work this past year has also provided second alleles of many UniformMu mutants.

Feasibility of using UniformMu maize mutants in forward-genetics approaches has been markedly increased by the development of Mu-seq protocols for use with multiplexed samples from mutants in gridded arrays.

Below and Gentry (Illinois AES)

More producers are achieving greater yields from crop management decisions based on the quantification of the roles of genetics, population, fertilizer, foliar protection, and other inputs for maize and soybean yield and biomass production from photosynthate utilization. Also, using tropical maize hybrids for bioenergy produced over 15% sugar in the stalks, which markedly reduces fermentation time compared to grain, and 0.22 g ethanol per gram total biomass.

Huber (Illinois AES/ARS)

Insights about the regulatory mechanisms controlling signaling pathways and metabolic enzymes may provide targets for manipulation in future studies to regulate growth, stomatal aperture, hormone signaling, and immune/stress signaling in crop plants to favor productivity.

Prasad and Aiken (Kansas AES)

We have improved our understanding of genetic variability and mechanisms associated with improved drought (water use efficiency and water availability) and high temperature tolerance. There was large genetic variability for root traits in wheat germplasm collections that could be utilized for improved resource capture and drought tolerance. Carbon isotope (13C) discrimination, measured in wheat grain developed under post-flowering drought, can help identify germplasm with drought stress avoidance. HT stress tolerant genotypes can be

identified by quantifying oxidative damage in pollen grains and by measuring pollen germination, seed-set percentage and grain yield at HT.

Moroney (Louisiana AES)

We are continuing to work to identify the components of the CO_2 concentrating mechanism of *Chlamydomonas reinhardtii*. To accomplish this, we generated 50,000 insertional mutants and conducted both a growth screen and a PCR-based screen on the mutant collection. We've identified strains with inserts in bestrophin, *NAR1.2* and genes encoding proteins thought to be participating in the photorespiratory pathway. We are also working to introduce some of the known CCM transport proteins into higher plants. This is part of the RIPE (Realizing Increased Photosynthetic Efficiency) project based out of the University of Illinois. I collaborate with the University of Illinois group as well as researchers at the Australian National University.

Benning (Michigan AES)

Insights into the regulation of carbon partitioning of primary photosynthate, i.e. sugars, into high energy storage compounds, e.g. triacylglycerol, in plants and algae will guide the engineering of vegetable oil food crops and novel biofuel crops. A mechanistic understanding how algal cells enter a quiescent state following nutrient starvation and exit it following refeeding will provide novel means to address the conundrum of cessation of growth during maximal accumulation of triacylglycerols in algae. Lipid remodeling under abiotic stress conditions is investigated as an important contributor to productivity in crop plants and algae.

Li (Mississippi AES)

The phytohormone abscisic acid plays a key role in stress-regulated carbon and nitrogen metabolism in plants. The abscisic acid function is mediated by abscisic acid-activated protein kinases and kinase-catalyzed protein phosphorylation. Thus, identification of phosphoproteins targeted by abscisic acid-activated protein kinases will reveal new components in abscisic acid signaling network.

Giroux (Montana AES)

The long term goal of the proposed research is to increase cereal agronomic yield. This goal may be achieved through further understanding of how source and sink strength influence plant yield. Here we focus on starch, as it is an important metabolite in both sink and source strength. Starch biosynthesis in the endosperm drives sink strength, whereas leaf starch is an important contributor of source strength and thus plant growth as excess photosynthate is stored as starch during the light period and remobilized during the dark period. This project is focused on understanding the importance of both leaf and seed starch biosynthesis in terms of metabolic, transcriptomic, and developmental factors currently limiting plant productivity in order to improve plant production.

Spreitzer (Nebraska AES)

A deeper understanding of the structure-function relationships of chloroplast ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), which catalyzes the rate-limiting step of photosynthesis, will identify targets for engineering an increase in CO_2 fixation. The green alga

Chlamydomonas reinhardtii, which is a well-developed model genetic organism for the study of photosynthesis, can be used as a host to facilitate the engineering of crop-plant Rubisco.

Stone (Nebraska AES)

Human DJ-1 has garnered great interest due to its implication in protecting cells against oxidative stress and consequently many different human diseases, including various cancers and neurodegenerative diseases. Little is known about the function of plant DJ-1-like proteins, but they are likely to mediate their functions in manners analogous to other superfamily members. A key structural feature of all DJ-1 superfamily members is that they form dimers (or higher oligomers) with differing interfaces, and these oligomers are essential for the function of these proteins. The unique gene structure of the plant DJ-1-like proteins strongly suggests that these plant proteins form a DJ-1-like "dimer" within a single polypeptide. If true, this will significantly simplify certain experiments, particularly the coimmunoprecipitation and/or yeast two-hybrid experiments to identify interacting macromolecules.

Weeks (Nebraska AES)

We have successfully expanded our earlier work on the use of the CRISPR/Cas9/sgRNA system for targeted gene knockout in higher plants. We have demonstrated highly efficient gene editing using Cas9/sgRNA in Arabidopsis and tobacco and have published data showing stable inheritance of the edited genes in T2- and T3-generation plants. More recently in collaboration with Dr. Bing Yang at Iowa State University we have produced vectors containing multiple single guide RNA genes that allow simultaneous knockout of four different genes and/or the creation of large chromosomal regions ranging from a 100 bp to 250 kbp. Finally, we have recently published results showing successful transient expression of the CRISPR/Cas9/sgRNA system in *Chlamydomonas reinhardtii*, but failure to obtain viable cells bearing permanent gene knockouts.

Cushman (Nevada AES)

Reiterative, transgressive selection strategies using buoyant density gradient centrifugation and fluorescence-activated cell sorting and flow cytometry have been used successfully to select and isolate algal strains with greater starch or lipid contents. These approaches represent potential alternatives to genetic engineering strategies for the alteration of microbial biofuel feedstock traits.

Crassulacean acid metabolism (CAM) is an alternative mode of photosynthesis that improves water-use efficiency. Current research is focused on characterizing the genome-scale requirement of CAM using comparative genomics approaches among taxonomically diverse CAM species within an evolutionary framework. Such information will be used to move CAM into C_3 crops and short-rotation forestry bioenergy crops and thereby improving their water-use efficiency.

Jang (Ohio AES)

Plant Arginine-Rich (RR)-Tandem CCCH Zinc Finger proteins (RR-TZFs) are powerful switches for hormone- and environmental factor-mediated plant growth and stress responses. Numerous reports have indicated that RR-TZF proteins can control plant size, flowering time, and enhance

biotic and abiotic stress responses via regulation of gene expression. Despite growing genetic evidences, the underlying molecular mechanisms are virtually unknown. We have demonstrated: 1) RR-TZF proteins are conserved across monocots to dicots; 2) RR-TZFs can target and degrade specific mRNAs; 3) RR-TZFs preferentially interact with stress responsive regulators.

Gillaspy (Virginia AES)

The sucrose non-fermenting related kinases (SnRKs) are major energy sensors in eukaryotes that impact global transcription programs, stress signaling and lifespan. Work on the functional consequences of expression of different SnRK1 genes and protein isoforms culminated this year. We were able to show that specific SnRK1 isoforms delay early plant development resulting in more biomass production later in development. A separate approach addressing potential low energy signaling molecules called inositol pyrophosphates also culminated in a recent publication. Inositol pyrophosphates are important players in eukaryotic energy and metabolic regulation. Characterization of two plant kinase genes involved in inositol pyrophosphate synthesis was reported.

Edwards and Okita (Washington AES)

C_4 plants have a CO_2 concentrating mechanism which reduces loss of CO_2 by photorespiration. Efforts are being made to identify how the C_4 system develops and requirements for C_4 function, in order to transfer C_4 traits into C_3 crops. During this report period information was obtained relative to these efforts.

During the reproductive stage, sink strength is dictated by the conversion of transported photoassimilates into starch and protein, the major storage reserves in many plant seeds. Current efforts 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.

Publications

1. Andorf, C.M., Kopylov, M., Dobbs, D., Koch, K.E., Stroupe, M.E., Lawrence, C.J., and Bass, H. (2014) G-quadruplex (G4) motifs in the maize (*Zea mays* L.) genome are enriched at specific locations in thousands of genes coupled to energy status, hypoxia, low sugar, and nutrient deprivation. JGG (J. Genetics and Genomics). (In press).
2. Bender, R.R., Haegele, J.W., and Below, F.E. (2014). Nutrient uptake, partitioning, and remobilization in modern soybean varieties. Agron J. In press
3. Bihmidine, S., Hunter, C.T., Johns, C.E., Koch, K.E., and Braun, D. (2013). Regulation of assimilate import into sink organs: Update on molecular drivers of sink strength. Frontiers in Plant Physiology 4:177 (15 pgs)
4. Blaby IK, Glaesener AG, Mettler T, Fitz-Gibbon ST, Gallaher SD, Liu B, Boyle NR, Kropat J, Stitt M, Johnson S, Benning C, Pellegrini M, Casero D, Merchant SS. 2013. Systems-level analysis of nitrogen starvation-induced modifications of carbon metabolism in a *Chlamydomonas reinhardtii* starchless mutant. Plant Cell, 25:4305-4323
5. Bogamuwa, S. and Jang, JC (2014). Tandem CCCH zinc finger proteins in plant growth, development, and stress response. *Plant and Cell Physiology* 55: 1367–1375. (Invited Review).
6. Borland AM, Hartwell J, Weston D, Schlauch KA, Tschaplinski TJ, Tuskan GA, Yang X, Cushman JC. (2014) Engineering Crassulacean acid metabolism to improve water-use efficiency. Trends Plant Sci. 19: 327-338. http://dx.doi.org/10.1016/j.tplants.2014.01.006. Video abstract for this article is available at: http://www.cell.com/trends/plant-science/home.
7. Borland AM, Wullschleger SD, Weston DJ, Hartwell J, Tuskan GA, Yang X, Cushman JC. (2014b) Climate-resilient agroforestry: Physiological responses to climate change and engineering of crassulacean acid metabolism (CAM) as a mitigation strategy. Plant Cell & Environ. *In press.*
8. Brueggeman, A.J., D. Kuehler, D. P. Weeks (2014) Evaluation of three herbicide resistance genes for use in genetic transformations and for potential crop protection in algae production. *Plant Biotechnology Journal* doi: 10:1111/pbi.12192
9. Cakir, B. A. Tuncel, S.-K. Hwang and T.W. Okita (2014) Increase of grain yields by manipulating starch synthesis. In: Starch: Metabolism, Structure and Function, Nakamura, Y. ed., Springer. In press.
10. Chen, M.H., Dien, B., Vincent, M.L., Below, F.E., and Singh. V. (2014). Effect of harvest maturity on carbohydrates for ethanol production from sugar enhanced temperate x tropical maize hybrid. Indust. Crops Products 60, 266-272.
11. Choe, E., Williams, M.W., Boydston, R.A., Huber, J.L., Huber, S.C., and Pataky, J.K. (2014). Photosystem II-inhibitors play a limited role in sweet corn response to 4-hydroxyphenyl pyruvate dioxygenase-inhibiting herbicides. Agron. J. 106: 1317-1323.

12. Cushman JC. (2014) Interview with John C. Cushman. Trends Plant Sci. 19: 274-275. DOI: http://dx.doi.org/10.1016/j.tplants.2014.03.003.

13. DePaoli HC, Borland AM, Tuskan GA, Cushman JC, Yang X. (2014) Synthetic biology as it relates to CAM photosynthesis: Challenges and opportunities. J. Exp. Bot. 65:3381-93. doi: 10.1093/jxb/eru038

14. Desai M, Rangarajan P, Donahue JL, Williams SP, Land ES, Mandal MK, Phillippy BQ, Perera IY, Raboy V, Gillaspy GE. (2014) Two inositol hexakisphosphate kinases drive inositol pyrophosphate synthesis in plants. Plant J. 80(4):642-53.

15. Djanaguiraman, M., Prasad, P.V.V., Murugan, M., Reddy, U.K. 2014. Physiological differences among sorghum (*Sorghum bicolor* L. Moench) genotypes under high temperature stress. Environmental and Experimental Botany 100, 43-54.

16. Djidonou, D., Lopiano, K., Zhao, X., Simonne, E.H., Erickson, J.E., and Koch, K.E. (2014) Estimating nitrogen nutritional crop requirements of grafted tomatoes under field conditions. Scientia Horticulturae (In press).

17. Doroshenk, K.A., L. Tian, A.J. Crofts, T. Kumamaru, T.W. Okita (2014) Identification and characterization of a novel RNA-binding protein involved in glutelin mRNA processing and localization. Plant Mol. Biol. 85:381-394. DOI 10.1007/s11103-014-0191-z

18. Gandin, A., Koteyeva, N.K., Voznesenskaya, E.V., Edwards, G.E., and Cousins, A.B. 2014 The acclimation of photosynthesis and respiration to temperature in the C_3-C_4 intermediate *Salsola divaricata*: Induction of high respiratory CO_2 release under low temperature. Plant Cell and Environment. DOI: 10.1111/pce.12345

19. Gao, H., D. A. Wright, T. Ling, Y. Wang, K. Horken, D. P. Weeks and M.H. Spalding (2014) TALE activation of endogenous genes in *Chlamydomonas reinhardtii* *Algal Research* (in press)

20. Haegele, J.W., Becker, R.J., Henninger, A.S., and Below, F.E. (2014). Row arrangement, phosphorus fertility, and hybrid contributions to managing increased plant density of maize. Agron J 106, 1838-1846.

21. Hathwaik, L.T., Cushman, J.C. (2014). Strain selection strategies for improvement of algal biofuel feedstocks. In: Biofuels and Bioenergy. Eds: John Bryant, Clive Butler, and John Love. Wiley-Blackwell, UK. *In press*

22. Hebbar, K.B., Rane, J., Ramana, S., Panwar, N.R., Ajay, S., Subba Rao, A., Prasad, P.V.V. 2014. Natural variation in the regulation of leaf senescence and relation to N and root traits in wheat. Plant and Soil 378, 99-112.

23. Hunter C.T., Suzuki M., Saunders J.W., Wu S., Tasi A., Avigne W.T., McCarty D.R., and Koch K.E. (2014) Phenotype to genotype using forward-genetic Mu-seq for identification and functional classification of maize mutants. Frontiers in Plant Science. 4 (545). DOI: 10.3389/fpls.2013.00545.

24. Hurlock AK, Roston RL, Wang K, Benning C. (2014) Lipid trafficking in plant cells.Traffic. 2014 Jun 16. doi: 10.1111/tra.12187.

25. Jiang, W., A. J. Brueggeman, K. M. Horken, T. M. Plucinak and D. P. Weeks (2014) Successful transient expression of Cas9/sgRNA genes in *Chlamydomonas reinhardtii*. *Eukaryotic Cell* Sep 19. pii: EC.00213-14. [Epub ahead of print]

26. Jiang, W., S. Cossey, J.N. Rosenberg, G.A. Oyler, B.J.S.C. Olson and D.P. Weeks (2014) A rapid live-cell ELISA for characterizing antibodies against cell surface antigens of *Chlamydomonas reinhardtii* and its use in isolating algae from natural environments with related cell wall components. *BMC Plant Biology* 14:244 DOI: 10.1186/s12870-014-0244-0

27. Jiang, W., B. Yang and D.P. Weeks (2014) Efficient CRISPR/Cas9-mediated gene editing in *Arabidopsis thaliana* and inheritance of modified genes in T2 and T3 generations. *PLOS ONE* 9:e99225. doi: 10.1371/journal.pone.0099225

28. Jungnick, N., Ma, Y., Mukherjee, B., Cronan, J.C., Speed, D.J., Susan M. Laborde, S.M., Longstreth, D.J., Moroney J.V. 2014. The Carbon Concentrating Mechanism in *Chlamydomonas reinhardtii* - Finding the Missing pieces. Photosynth. Res. 121:159-173 http://link.springer.com/article/10.1007/s11120-014-0004-x/fulltext.html

29. Kadam, N.N., Xiao, G., Melgar, R.J., Bahuguna, R.N., Quinones, C., Tamilselvan, A., Prasad, P.V.V., Jagadish, S.V.K. 2014. Agronomic and physiological response to high temperature, drought and elevated carbon dioxide interactions in cereals. Advances in Agronomy 127, 111-156.

30. Kimberlin, A.N., Majumder, S., Han, G., Chen, M., Cahoon, R.E., Stone, J.M., Dunn, T.M., and Cahoon, E.B. (2013). Arabidopsis 56-amino acid serine palmitoyltransferase-interacting proteins stimulate sphingolipid synthesis, are essential, and affect mycotoxin sensitivity. *Plant Cell* 25: 4627-4639.

31. Kirst, H., and Melis, A. (2014). The chloroplast *Signal Recognition Particle* pathway (CpSRP) as a tool to minimize chlorophyll antenna size and maximize photosynthetic productivity. Biotechnology Advances 32, 66–72. DOI: 10.1016/j.biotechadv.2013.08.018.

32. Kirst, H., Formighieri, C., and Melis, A. (2014). Maximizing photosynthetic efficiency and culture productivity in cyanobacteria upon minimizing the phycobilisome light-harvesting antenna size. Biochim Biophys Acta - Bioenergetics 1837, 1653-1664. DOI: 10.1016/j.bbabio.2014.07.009.

33. Koteyeva, N.K., Voznesenskaya, E.V., Cousins, A.B. and Edwards, G.E. 2014 Differentiation of C_4 photosynthesis along a leaf developmental gradient in two *Cleome* species having different forms of Kranz anatomy. J Exp Bot 65, 3525-3541 doi:10.1093/jxb/eru042

34. Macho, A.P., Schwessinger, B., Ntoukakis, V., Brutus, A., Segonzac, C., Roy, S., Kadota, Y., Oh, M.-H., Sklenar, J., Derbyshire, P., Lozano-Duran, R., Malinovsky, G., Monaghan, J., Menke, R.L., Huber, S.C., He, S.Y., and Zipfel, C. (2014). A bacterial tyrosine phosphatase inhibits plant pattern recognition receptor activation. Science 343: 1509-1512.

35. Mahama, G.Y., Prasad, P.V.V., Mengel, D.B., Tesso, T.T. 2014. Influence of nitrogen fertilizer on growth and yield of sorghum hybrids and inbred lines. Agronomy Journal 106, 1623-1630.

36. Memmola, M., Mukherjee, B., Moroney, J.V., Giordano M. 2014. Carbon allocation and element composition in four Chlamydomonas mutants defective in genes related to the CO_2 Concentrating Mechanism. Photosynth. Res. 121:201-211 http://link.springer.com/article/10.1007/s11120-014-0005-9/fulltext.html

37. Moroney, J.V., Wee, J.L. 2014. CCM8: The Eighth International Symposium on Inorganic Carbon Uptake by Aquatic Photosynthetic Organisms. Photosynth. Res. 121:107-110 http://link.springer.com/article/10.1007/s11120-013-9965-4/fulltext.html

38. Narayanan S, Aiken RM, Prasad PVV, Xin Z, Paul G, Yu J. 2014. A simple quantitative model to predict leaf area index in sorghum. Agronomy Journal 106, 219-226.

39. Narayanan, S., Mohan, A., Gill, K.S., Prasad, P.V.V. 2014. Variability of root traits in spring wheat germplasm. PLoS One 9(6), e100317.

40. Narayanan, S., Prasad, P.V.V. 2014. Characterization of a spring wheat association mapping panel for root traits. Agronomy Journal 106, 1593-1604.

41. Ocheltree, T.R., Nippert, J.B., Kirkham, M.B., Prasad, P.V.V. 2014. Partitioning hydraulic resistance in sorghum bicolor leaves reveals unique correlations with stomatal conductance during drought. Functional Plant Biology 41, 25-36.

42. Ocheltree, T.R., Nippert, J.B., Prasad, P.V.V. 2014. Stomatal response to changes in vapor pressure deficit reflect tissue-specific differences in hydraulic conductance. Plant Cell and Environment 37, 132-139.

43. Oh, M.-H., Wang, X., Kim, S.Y., Wu, X., Clouse, S.D. and Huber, S.C. (2014). The Carboxy-terminus of BAK1 regulates kinase activity and is required for normal growth of Arabidopsis. Front. Plant Sci,doi: 10.3389/fpls.2014.00016.

44. Paul, G., Gowda, P.H., Prasad, P.V.V., Howell, T.A., Aiken, R.M. 2014. Investigating the influence of roughness length for heat transport (zoh) on performance of SEBAL in semi-arid irrigated and dryland agricultural systems. Journal of Hydrology 209, 231-234.

45. Qu, J., Kang, S. G., Musier-Forsyth, K., and Jang J. C. (2014). *Arabidopsis thaliana* tandem zinc finger 1 (AtTZF1) in RNA binding and decay. *Plant Journal* 78: 452-467.

46. Riekhof WR, Naik S, Bertrand H, Benning C, Voelker DR. 2014. Phosphate starvation in fungi induces the replacement of phosphatidylcholine with the phosphorus-free betaine lipid diacylglyceryl-N,N,N-trimethylhomoserine. Eukaryot Cell. 13:749-5

47. Rosnow, J., Edwards, G.E. Roalson, E.H. 2014 Evolutionary Positive selection of Kranz and nonKranz C_4 phosphoenolpyruvate carboxylase amino acids in Suaedoideae (Chenopodiaceae). J Exp Bot 65, 3595-3607 doi:10.1093/jxb/eru053

48. Rosnow, J., Yerramsetty, P.K., Berry, J.O., Okita, T.W. and Edwards, G.E. 2014 Exploring mechanisms linked to differentiation and function of dimorphic chloroplasts in the

single cell C4 species *Bienertia sinuspersici*. BMC Plant Biology 14, 34 doi:10.1186/1471-2229-14-34

49. Roston RL, Hurlock AK, Benning C. (2014). Plastidic ABC transporters. In Plant ABC Transporters, ed. Geisler M. (Springer-Verlag, Berlin Heidelberg) in press.
50. Roston RL, Wang K, Kuhn LA, Benning C. 2014. Structural determinants allowing transferase activity in SENSITIVE TO FREEZING 2, classified as a family I glycosyl hydrolase. J Biol Chem. 289:26089-106.
51. Ruffo, M.L., Gentry, L.F., Henninger, A.S., Seebauer, J.R., and Below, F.E. (2014). Evaluating management factor contributions to reduce corn yield gaps. Agron J. In press
52. Schlosser, A.J., J.M. Martin, B.S. Beecher, and M.J. Giroux. Enhanced rice growth is conferred by increased leaf ADP-glucose pyrophosphorylase activity. Journal of Plant Physiology & Pathology, accepted September 27, 2014, in press.
53. Silvera K, Winter K, Rodgriguez BL, Albion, RL, Cushman JC. (2014) Multiple isoforms of phophoenolpyruvate carboxylase in the Orchidacea (subtribe Oncidiinae): implications for the evolution of crassulacean acid metabolism. J. Exp. Bot. 65:3623-36. doi: 10.1093/jxb/eru234
54. Singh, P., Swamikannu, N., Traore, S.P., Boote, K.J., Rattunde, H.F.W., Prasad, P.V.V., Singh, N.P., Srinivas, K., Bantilan, C. 2014. Quantifying potential benefits of drought and heat tolerance in rainy season sorghum for adapting to climate change. Agricultural and Forest Meteorology185, 231-234.
55. Soares, J.S.M., Gentile, A., Scorsato, V., da C. Lima, A., Kyota, E., dos Santos, M.L., Piattoni, C.V. Huber, S.C., Aparicio, R. and Menossi, M. (2014). Oligomerization, membrane association and in vivo phosphorylation of sugarcane UDP-glucose pyrophosphorylase. J Biol Chem., in press.
56. Stutz, S.S., Edwards, G.E., Cousins, A.B. 2014 Single cell C_4 photosynthesis: efficiency and acclimation of *Bienertia sinuspersici* to growth under low light. New Phyto 202, 220-232.
57. Tian, L. and T.W. Okita (2014) mRNA-based protein targeting to the endoplasmic reticulum and chloroplasts in plant cells. Curr. Op. Plant Biol. 22:77-85. DOI: 10.1016/j.pbi.2014.09.007 (http://www.sciencedirect.com/science/article/pii/S1369526614001289)
58. Tsai CH, Warakanont J, Takeuchi T., Sears BB, Moellering ER, Benning C. 2014.The protein COMPROMISED HYDROLYSIS OF TRIACYLGLYCEROLS 7 (CHT7) acts as a repressor of cellular quiescence in Chlamydomonas. Proc Natl Acad Sci U S A, in press. doi: 10.1073/pnas.1414567111.
59. Tuncel, A, J. Kawaguchi, Y. Ihara, Y. Yoshida, H. Matsusaka, A. Nishi, T. Nakamura S. Kuhara, H. Hirakawa, Y. Nakamura, B. Cakir, A. Nagamine, T.W. Okita*, S.-K. Hwang*, and H. Satoh (2014) The rice endosperm ADP-glucose pyrophosphorylase large subunit is essential for optimal catalysis and allosteric regulation of the heteotetrameric enzyme. Plant Cell Physiol. 55(6):1169-83. doi: 10.1093/pcp/pcu057

60. Tuncel, A., B. Cakir, S.-K. Hwang and T.W. Okita (2014) Redox-regulation of the rice endosperm ADP-glucose pyrophosphorylase occurs through disulfide formation in the large subunit and not the small subunit as evident for the leaf and tuber enzymes. FEBS J. In press. doi:10.1111/febs.13041

61. Von Caemmerer, S., Edwards, G.E., Koteyeva, N., and Cousins, A. 2014 Single cell C_4 photosynthesis in aquatic and terrestrial plants: a gas exchange perspective. Aquatic Botany. 118, 71-80.

62. Williams SP, Rangarajan P, Donahue JL, Hess JE, Gillaspy GE. (2014) Regulation of Sucrose non-Fermenting Related Kinase 1 genes in *Arabidopsis thaliana*. Front Plant Sci. J0;5:324.

63. Yang, Y., A. J. Crofts, N. Crofts and T.W. Okita (2014) Multiple RNA binding protein complexes interact with the cis-localization RNA zipcode sequences of prolamine RNAs. Plant Physiol. 164:1271-1282. www.plantphysiol.org/cgi/doi/10.1104/pp.113.234187

64. Zhou, H., B. Liu, D.P. Weeks, M.H. Spalding, B.Yang (2014) Large chromosomal deletions and heritable small genetic changes induced by CRISPR/Cas9 in rice. *Nucleic Acids Research* Sept. 8. pii: gku806. [Epub ahead of print]