

NC-1168 Regulation of Photosynthetic Processes (Rev. NC-1142) Final Report

Period Covered: 10-1-2007 to 09-30-2012

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Executive Summary

The North Central Regional Group continued to work together on diverse aspects of photosynthesis over the 2007-2011-project period. Participating scientists sustained a high degree of productivity by publishing an average of 80 peer-reviewed scientific research reports and reviews annually during the duration of the project. The NC-1168 Group maintained a relatively constant membership with an average of 28 project participants during 2007-2011 with an average meeting participation rate of 41% of its individual membership over five years. Project meetings were held in five different venues over the course of the project including Nebraska NAES (2007), USDA-ARS Maricopa, AZ (2008), Ohio NAES (2009), Virginia NAES (2010), and Michigan NAES (2011). The interactions within this group have led to multiple research collaborations that have resulted in synergistic interactions and research discoveries. The group was well funded and garnered more than \$10M in extramural support over the project period.

Scientific Summary

Photosynthesis converts sunlight into plant biomass that is comprised of biochemical energy storage forms such as sugars, complex polysaccharides, lipids, amino acids and a wide variety of other compounds. Thus, understanding photosynthetic processes and the environmental factors, such as extreme heat or drought events, which negatively influence the agricultural production of food, fiber, and biofuel, is critical for maintaining the well being of human societies. The project participant's research interests spanned a wide range of organizational complexity from single molecules, macromolecular complexes, cells, leaves, whole plants, to field canopies. The group placed specific emphasis on understanding the signaling pathways triggered by abiotic stress (e.g., heat, cold, drought, and salinity), and the effects environmental insults have on photosynthetic efficiency and plant productivity, as well as nitrogen and water use efficiency. The research efforts of the project participants were organized into four major research areas: 1) plastid function and intracellular communication, 2) photosynthetic capture and photorespiratory release of CO₂, 3) mechanisms regulating photosynthate partitioning, and 4) developmental and environmental limitations to photosynthesis.

Research into plant function and intracellular communication focused on characterizing variegation mutants in *Arabidopsis* to explore the role of a plastid terminal oxidase and mitochondrial alternative oxidase in normal chloroplast biogenesis, chlororespiration, and light-to-dark transitions to poise the redox state of the plastidic electron transport chain. A second area of highly collaborative and synergistic plastid research involved the crystallization, structural characterization and improvement of Rubisco Activase to achieve higher thermotolerance of photosynthesis, determination of the mechanisms of redox regulation of Rubisco Activase isoforms in *Arabidopsis*, *Camelina* and tobacco, and characterization of the interactions between Rubisco Activase and Rubisco using *Chlamydomonas* Rubisco mutants. A third area of plastid research centered upon the functional roles of three *Arabidopsis* homologues to the Human DJ-1 protein, which function as an obligate homodimer to protect against reactive oxygen species-mediated programmed cell death, and are essential for seedling viability.

The project participants conducted several projects focused on photosynthetic capture and photorespiratory release of CO₂. First, the carbonic anhydrase gene family in *Chlamydomonas* was characterized and the function of specific plastid-localized gene family members was characterized by insertional mutagenesis resulting in slow-growth phenotypes. Second, a collaborative project elucidated the structural basis of Rubisco catalytic efficiency and CO₂/O₂ specificity using the *Chlamydomonas* Rubisco as a model. The structural requirements for localization to the pyrenoid and Rubisco-Rubisco Activates interactions are also being investigated. Third, TAL Effector Nuclease (TALEN) technology for targeted gene knockout in eukaryotic organisms was developed in a collaborative effort. The successful application of TALENs was demonstrated by causing inactivation of the disease susceptibility gene and the production of rice plants resistant to bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo).

Another major area of investigation by the project is the understanding of mechanisms that regulate photosynthate partitioning. One major research program involved the development of methods to increase oil biosynthesis in vegetative tissues. In one example, ectopic expression of the WRI transcription factor from *Arabidopsis* along with concurrent repression of ADP-glucose pyrophosphorylase increased TAG accumulation by dry weight 5-10% in *Brassica napus* leaves. In a related project,

overexpression of diacylglycerol acyltransferase type two (DGTT) enzymes from *Chlamydomonas reinhardtii* in *Arabidopsis* increased TAG content of leaves along with the production of very long chain fatty acids (C27-C33 alkanes). Also, studies of the accumulation of TAGs in response to nitrogen deprivation in the model alga *Chlamydomonas* revealed that galactoglycerolipid pools serve as a major source of fatty acids esterified into triacylglycerols following N deprivation. Investigations into the function of C2H2 Tandem Zinc Finger (TZF) family of transcription factors revealed that they are positively regulated by ABA and negatively regulated by GA and when over-expressed resulted in compact rosettes and enhanced stress tolerance phenotypes in *Arabidopsis*. Elucidation of the role of sucrose non-fermenting related kinases (SnRKs) revealed that these are major energy sensors in eukaryotes that impact global transcription programs, stress signaling and lifespan. Mutagenesis of myo-inositol oxygenase (MIOX) genes revealed that MIOX catabolism in shoots might influence root growth responses under low energy/nutrient conditions. Related work on calcium-dependent protein kinases (CPKs) revealed that certain members of this gene family can autophosphorylate Tyr residues, confirming that they possess dual specificity; however, this activity is rare in CPKs. Collaborative research also revealed for the first time that calmodulin (CaM) can bind *in vitro* to a predicted high affinity CaM binding site within the variable N-terminal domain of CPK-beta from soybean. Bacterial protein substrate specificity was also studied using an *E. coli*-based transphosphorylation assay and appeared to correlate with the intrinsic substrate specificity of the kinase assayed, at least for Thr and Tyr sites. Investigation of mutants that affect photosynthate partitioning and sugar signaling in maize revealed that strigolactones (SLs) control lateral branching by regulation of the Tb1 transcription factor. Mutation of Carotenoid Cleavage Dioxygenase 8 (CCD8), a gene essential for SL biosynthesis, resulted in increase branching and reduced stem diameter, and delayed adventitious root formation. In addition, mutation of a cellulose-synthase-like-1 (*csld1*) gene resulted in plants that accumulated 50% less biomass arising mainly from ineffective cross-wall formation revealing the impact of early-development partitioning of photosynthate to cell wall material. Lastly, mutations in photosynthetic electron transport and Calvin-Benson cycle enzymes revealed that starch and sucrose partitioning was established by phosphoglucomutase (pgm), which when mutated has little to no carbon flux to starch as predicted; however, when triose phosphate translocator (tpt) was mutated, plants had relatively more sucrose than predicted resulting in novel hypotheses about how carbon export from chloroplasts during the day is regulated, how limits on photosynthesis imposed by feedback effects might be lifted, and how sucrose/starch partitioning in photosynthesis affects plant development through sugar signaling.

The fourth focal area is improved understanding of the developmental and environmental limitations on photosynthesis. For example, increasing surface temperatures is expected to result in large increases in the rates of drought-associated evapotranspiration. Surveys of wild wheat accessions have identified *Aegilops speltoides* and *A. geniculata* accessions with greater heat tolerance and surveys of different sorghum genotypes have identified lines with differences in their sensitivity to elevated water vapor pressure deficit due to different strategies for soil water conservation in water-deficit environments through modulation of transpiration rates. Field-scale research showed that continuous corn rotations sequestered an average of 62% more C in intermediate- and long-term soil carbon pools than corn-soybean rotations, but that continuous corn treatments produced, on average, 13 bu a⁻¹ less than corn-soybean rotations. Advanced nutrition (N, P, S, and Zn applications) increased corn yields significantly. Increasing plant density by 40% increased the effects of drought stress, especially in continuous corn systems. High throughput metabolomic studies on resurrection plants have revealed that desiccation tolerance is associated with the accumulation of sucrose, mono- and polysaccharides, γ -glutamyl amino acids, citrulline, and nucleotide catabolism products (e.g., allantoin) for nitrogen remobilization or ROS scavenging, and potent antioxidant or UV-protective or antioxidant compounds, such as 3-(3-hydroxyphenyl)propionate, apigenin and naringenin. Further research has shown that at least six Cyclic Nucleotide Gated Channels (CNGCs) are expressed in pollen of *Arabidopsis* and that several are essential for pollen viability including CNGC18 and 7/8 with some genes (e.g., CNGC16) showing conditional reductions in pollen viability under a cold night and hot day temperature stress regime suggesting that CNGCs might participate in stress-adaptive responses. Investigations into the function of a family of 12 lipid flippases (ALAs) belonging to a unique subfamily of P-type ATPases revealed that several family members likely function to regulate the abundance of specific lipids in the membrane under the control of CPKs. Lastly, collaborative investigations into the improvement of the Rubisco activase to achieve higher thermotolerance of photosynthesis are ongoing.