Enhancing chloroplast-mitochondrial metabolic networks to improve photosynthesis and crop yields

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Project Goals:

Increased photosynthetic efficiency has the potential to address the predicted shortfalls in agricultural production by the middle of this century. Photosynthesis is highly sensitive to varying environmental conditions, including temperature, water and nutrient availability. Metabolic interactions between chloroplasts and mitochondria impact photosynthesis by redistributing and decreasing excess reducing equivalents and optimizing CO_2 fixation via photorespiratory metabolism. We studied components of the carbon concentrating mechanisms (CCMs) of aquatic algae to gain insight into the chloroplast-mitochondrial metabolic networks that function to optimize photosynthesis under limiting environmental conditions. We describe improved carbon assimilation, increased water and nitrogen use efficiency, and higher seed yields in the oil seed crop, *Camelina sativa*, expressing LIP36, a mitochondrial CCM component of *Chlamydomonas reinhardtti*. Our results suggest that LIP36 participates in redistributing metabolic intermediates to balance photosynthetic and photorespiratory metabolism, thereby maintaining plant productivity under non-ideal growth conditions.

Abstract:

The projected increases in global population growth, and the desire to transition to a sustainable, bio-based economy are resulting in ever increasing demands on agricultural productivity. One major limitation to increasing crop yields is the inefficiency of photochemical conversion of light to fixed carbon during photosynthesis. For example, the oxygenation of ribulose-1,5-bisphosphate (RuBP) catalyzed by Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) competes with CO_2 fixation and leads to a significant energy cost, generation of harmful reactive oxygen species, and the net loss of fixed carbon to the plant (Walker et al., 2016). Furthermore, the toxic products of oxygenation must be recycled via photorespiration, accounting for a >40% decrease in the efficiency of carbon fixation in C_3 photosynthesis, the pathway of carbon fixation used by the majority of crops and about 85% of all terrestrial plant species (Walker et al., 2016). Thus, strategies to balance the flux through photosynthetic and photorespiratory metabolism, while maintaining resilience to changing environmental conditions have the potential to broadly impact crop yields.

Chloroplasts and mitochondria serve complementary functions in plant growth through their primary roles in photosynthesis and respiration, respectively. In addition to their participation in energy generation, photosynthesis and respiration act in concert as components of an integrated network of metabolism to maintain cellular redox balance, optimize photosynthetic carbon capture, coordinate carbon and nitrogen metabolism, and mediate specific aspects of cellular stress signaling (Igamberdiev et al., 2018). As a consequence, knowledge of the interdependence and

coordinate control of chloroplast and mitochondrial metabolism is required to understand how plants optimize growth under both normal and stress conditions. In turn, these studies will facilitate strategies aimed at optimizing plant resilience and agricultural yields in the face of changing environmental conditions.

The primary example of chloroplast-mitochondrial metabolic integration is photorespiration. The conversion of glycine to serine in mitochondria by the combined action of glycine decarboxylase and serine hydroxymethyltransferase is a required step in recycling the toxic product of the oxygenation reaction catalyzed by ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco)(Bauwe et al., 2010) via photorespiratory metabolism. Photorespiration is estimated to account for upwards of 40% of flux through central carbon metabolism in C_3 photosynthesis, the pathway used by the majority of crops and about 85% of all terrestrial plant species (Ehleringer et al., 1991;Walker et al., 2016;Bauwe et al., 2010). Although photorespiration is often viewed as a non-productive process, photorespiratory metabolism is essential in plants, and higher photosynthetic rates and biomass production are observed by increasing the levels of glycine decarboxylase activity, demonstrating the positive link between mitochondrial photorespiratory metabolism, photosynthesis, and growth (Timm et al., 2015;Timm et al., 2012).

Photosynthesis and CO_2 assimilation also are affected by mutations that alter the activity of select enzymes in the tricarboxylic acid (TCA) cycle or that impact complex I of the mitochondrial electron transport chain (Carrari et al., 2003;Araújo et al., 2011;Nunes-Nesi et al., 2007;Nunes-Nesi et al., 2005;Bartoli et al., 2005;Dutilleul et al., 2003;Gandin et al., 2012;Chai et al., 2010), implicating specific aspects of respiratory metabolism in maintaining photosynthesis. Conversely, alterations in photosynthetic cyclic electron flow (Dang et al., 2014;Larosa et al., 2018) or NADH utilization in chloroplasts (Wu et al., 2015) result in significant changes to mitochondrial metabolic activity. The physiological role of these links is not fully understood, but has been attributed to an essential contribution of mitochondria to controlling the levels and distribution of reducing equivalents across cellular compartments and providing TCA cycle intermediates as carbon skeletons for anabolic metabolism and nutrient assimilation (Araújo et al., 2011;Nunes-Nesi et al., 2008).

Algal carbon concentrating mechanisms (CCMs)(Giordano et al., 2005) provide an excellent system to investigate cellular responses to the limitations imposed on photosynthesis and associated metabolism by environmental conditions. Many algae are regularly subjected to CO_2 limitation because of their aquatic habitats, and in response, have evolved low-CO_2 inducible CCMs to concentrate CO_2 at the Rubisco active site, and reconfigure metabolic networks to maintain CO_2 fixation and growth (Meyer et al., 2013). In *Chlamydomonas reinhardtii* nearly one-third of all nonoverlapping genes in the genome undergo significant changes in expression when the CCMs are induced under low-CO_2 conditions, and more than a thousand genes are significantly upregulated (Brueggeman et al., 2012;Meyer et al., 2013;Zhu et al., 2010). Two of the most highly induced genes of the chlamydomonas CCM encode low-CO_2-inducible proteins with a molecular mass of 36 kD (LIP36)(Moroney et al., 1991;Ramazanov et al., 1993). The LIP36 proteins, LIP36G1 and LIP36G2, are 96% identical and appear to be functionally redundant. They are predicted to be members of the mitochondrial carrier protein family, and LIP36-GFP fusion constructs localize to mitochondria in chlamydomonas and transgenic tobacco (Atkinson et al., 2016). Suppression of LIP36 expression by RNA interference demonstrated that they are essential for the growth of chlamydomonas under low CO_2 conditions (Pollock et al., 2004), consistent with

an essential role for mitochondrial metabolism in optimizing photosynthesis under limiting environmental conditions.

To gain insight into the role of LIP36 proteins, we expressed LIP36G1 in the oilseed crop plant, *Camelina sativa* (camelina). We demonstrate that expression of LIP36G1 significantly increased CO_2 assimilation and seed yields in camelina relative to control plants under limiting environmental conditions. Metabolic analysis suggests that LIP36 has a role in modifying mitochondrial metabolism during photosynthesis to optimize the balance between photosynthesis and photorespiration, and to provide intermediates for anabolic metabolism. The LIP36 lines also exhibited increased water use efficiency due to decreased stomatal conductance, demonstrating that it is possible to break the trade-off between water use efficiency and yield. Our results provide new insight into the interdependence of organellar metabolic networks in photosynthetic metabolism and illustrate the potential positive effects of engineering components of the algal CCM into vascular plants on crop productivity.

References

Araújo, W.L., et al., Plant, Cell & Environment, 2011. **35**(1): p. 1-21.
Araújo, W.L., et al., The Plant Cell, 2011. **23**(2): p. 600.
Atkinson, N., et al., Plant Biotechnol J, 2016. **14**(5): p. 1302-15.
Bartoli, C.G., et al., Journal of Experimental Botany, 2005. **56**(415): p. 1269-1276.
Bauwe, H., M. Hagemann, and A.R. Fernie, Trends in Plant Science, 2010. **15**(6): p. 330-336.
Brueggeman, A.J., et al., Plant Cell, 2012. **24**(5): p. 1860-75.
Carrari, F., et al., Plant physiology, 2003. **133**(3): p. 1322-1335.
Chai, T.-T., et al., Plant physiology, 2010. **152**(3): p. 1638-1649.
Dang, K.-V., et al., The Plant Cell, 2014. **26**(7): p. 3036.
Dutilleul, C., et al., Plant physiology, 2003. **131**(1): p. 264-275.
Ehleringer, J.R., et al., Trends Ecol Evol, 1991. **6**(3): p. 95-9.
Gandin, A., et al., Plant and Cell Physiology, 2012. **53**(9): p. 1627-1637.
Giordano, M., J. Beardall, and J.A. Raven, Annu Rev Plant Biol, 2005. **56**: p. 99-131.
Igamberdiev, A.U. and N.V. Bykova, Free Radic Biol Med, 2018. **122**: p. 74-85.
Larosa, V., et al., Plant Physiology, 2018. **176**(3): p. 2305.
Meyer, M. and H. Griffiths, J Exp Bot, 2013. **64**(3): p. 769-86.
Moroney, J.V. and C.B. Mason, Can J Bot, 1991. **69**(5): p. 1017-1024.
Nunes-Nesi, A., et al., The Plant Journal, 2007. **50**(6): p. 1093-1106.
Nunes-Nesi, A., et al., Plant physiology, 2005. **137**(2): p. 611-622.
Nunes-Nesi, A., et al.,. Journal of Experimental Botany, 2008. **59**(7): p. 1675-1684.
Pollock, S.V., et al., Plant Mol Biol, 2004. **56**(1): p. 125-32.
Ramazanov, Z., et al., Plant Physiol, 1993. **101**(4): p. 1195-1199.
Timm, S., et al., FEBS Letters, 2012. **586**(20): p. 3692-3697.
Timm, S., et al.,. The Plant Cell, 2015. **27**(7): p. 1968.
Walker, B.J., et al., Annu Rev Plant Biol, 2016. **67**: p. 107-29.
Wu, J., et al., Cell Research, 2015. **25**: p. 621.
Zhu, X.G., S.P. Long, and D.R. Ort, Annu Rev Plant Biol, 2010. **61**: p. 235-61.

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