Integration of a Synthetic CO_2 Fixation Cycle into *Camelina sativa*

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Project Goals: To overcome the limitations of photosynthetic CO_2 fixation via Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase (RuBisCO) in plants, we created a RuBisCO-independent synthetic CO_2 fixation cycle based on enzymes from bacterial autotrophs. This condensed, reversed CO_2 fixation cycle consists of 5 enzymes expressed in the nucleus and imported into chloroplasts to generate glyoxylate from succinate (1). We show here the integration of partial and complete crTCA cycle enzyme in Camelina sativa chloroplast and its effect on physiology and gene expression.

Abstract:

Photosynthetic CO_2 fixation is catalyzed by Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase (RuBisCO), the most abundant enzyme on Earth. It's high abundance in plant chloroplasts is necessary due to its very low activity and specificity for CO_2. Attempts to improve the activity or specificity of RuBisCO have yielded little progress so far. We have focused on engineering a RuBisCO independent CO_2 fixation cycle into the chloroplast of *Camelina sativa* to increase overall CO_2 assimilation. This synthetic CO_2 fixation cycle is based on enzymes from autotropic bacteria utilizing a reverse TriCarboxylic Acid (TCA) cycle to fix CO_2. The minimal condensed reverse TCA cycle (crTCA) consists of 5 bacterial enzymes that generate glyoxylate from succinate and CO_2/bicarbonate.

We have shown that this engineered crTCA cycle can assimilate CO_2 *in vitro*. After codon-optimization, we were able to show that these enzymes can be expressed in plants. The genes were transformed into the nucleus as fusions containing chloroplast targeting sequences. Chloroplast-localized crTCA enzymes showed activity after purification.

In this study, stable, chloroplast-localized expression of the crTCA cycle in *Camelina sativa* is used to assess changes in photosynthetic parameters. Transgenic crTCA lines have increases in CO_2 assimilation rates under elevated CO_2 levels, greater efficiency in electron usage, and

differences in morphology compared to WT plants. To identify mechanisms beyond the changes CO_2 fixation, we carried out comparative transcriptome analysis from leaf material of transgenic camelina plants expressing the full or partial crTCA cycles with null segregant and empty vector lines. Using differential gene expression analysis, we were able distinguish distinct patterns between the different genotypes. Network analysis identified correlations between the expression of individual crTCA enzymes with changes in specific *Camelina* gene clusters.

While at least parts of the crTCA cycle are apparently functioning in assimilating CO_2, one of the major hurdles is the high abundance of RuBisCO, that competes with the comparatively lower abundance of the crTCA cycle enzymes. We are currently evaluating the full potential of the crTCA cycle in vivo by reducing the endogenous RuBisCO protein using an antisense approach.

References

1. **Bar-Even A., et al.** 2010. Design and analysis of synthetic carbon fixation pathways. *PNAS.* DOI: 10.1073/pnas.0907176107

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