

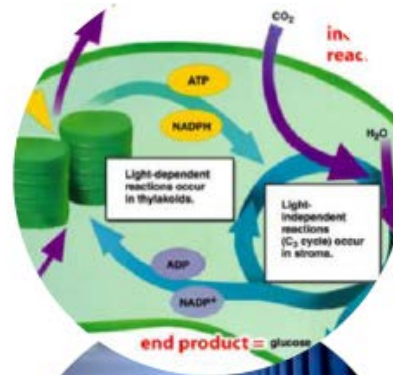
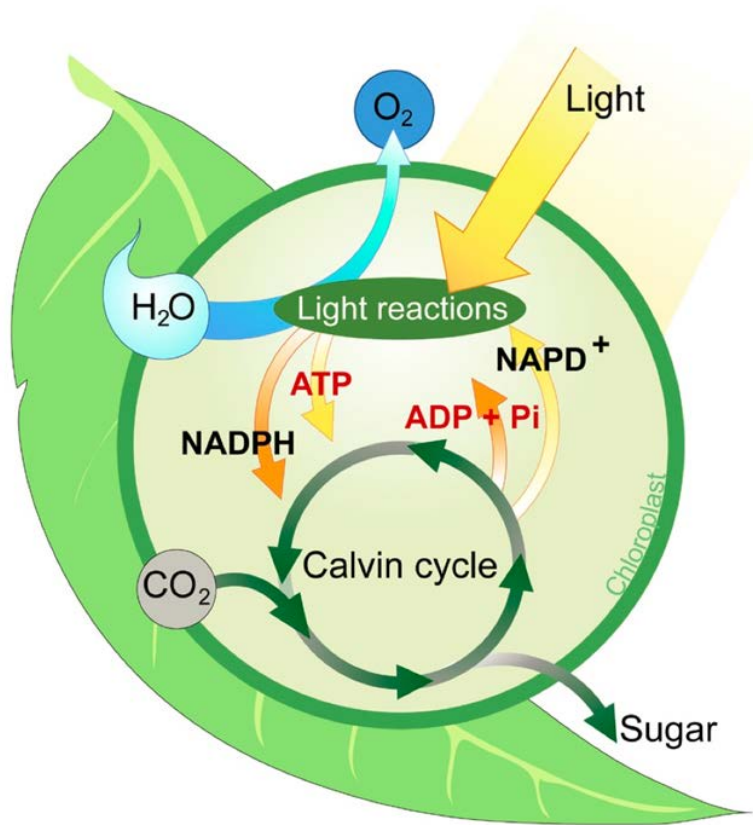
# Transgenic Opportunities to Enhance Photosynthetic Efficiency



**Lisa Ainsworth**

**USDA ARS Global Change and Photosynthesis  
Research Unit**

# Why improve photosynthesis?



Photosynthetic process is known in great detail



High performance computing for modeling the photosynthetic process

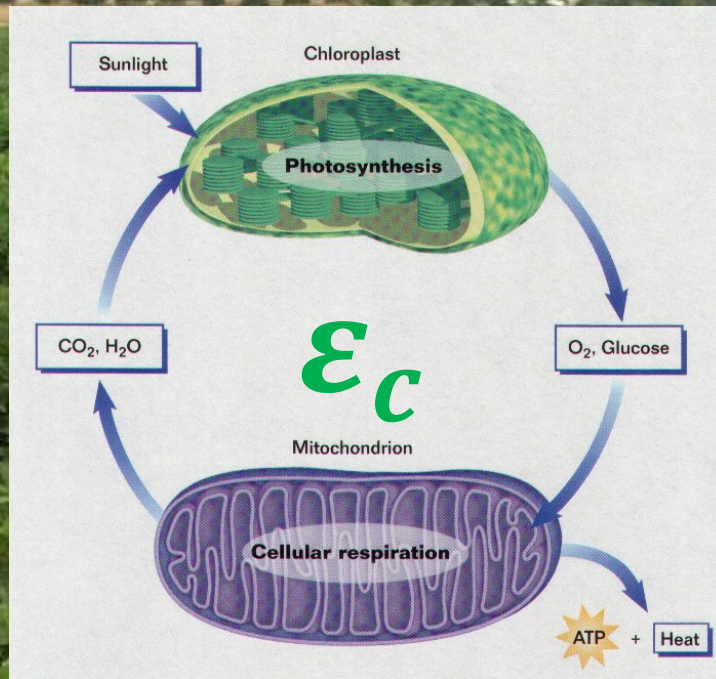


Crop transformation is getting more efficient

# Physiological drivers of yield potential

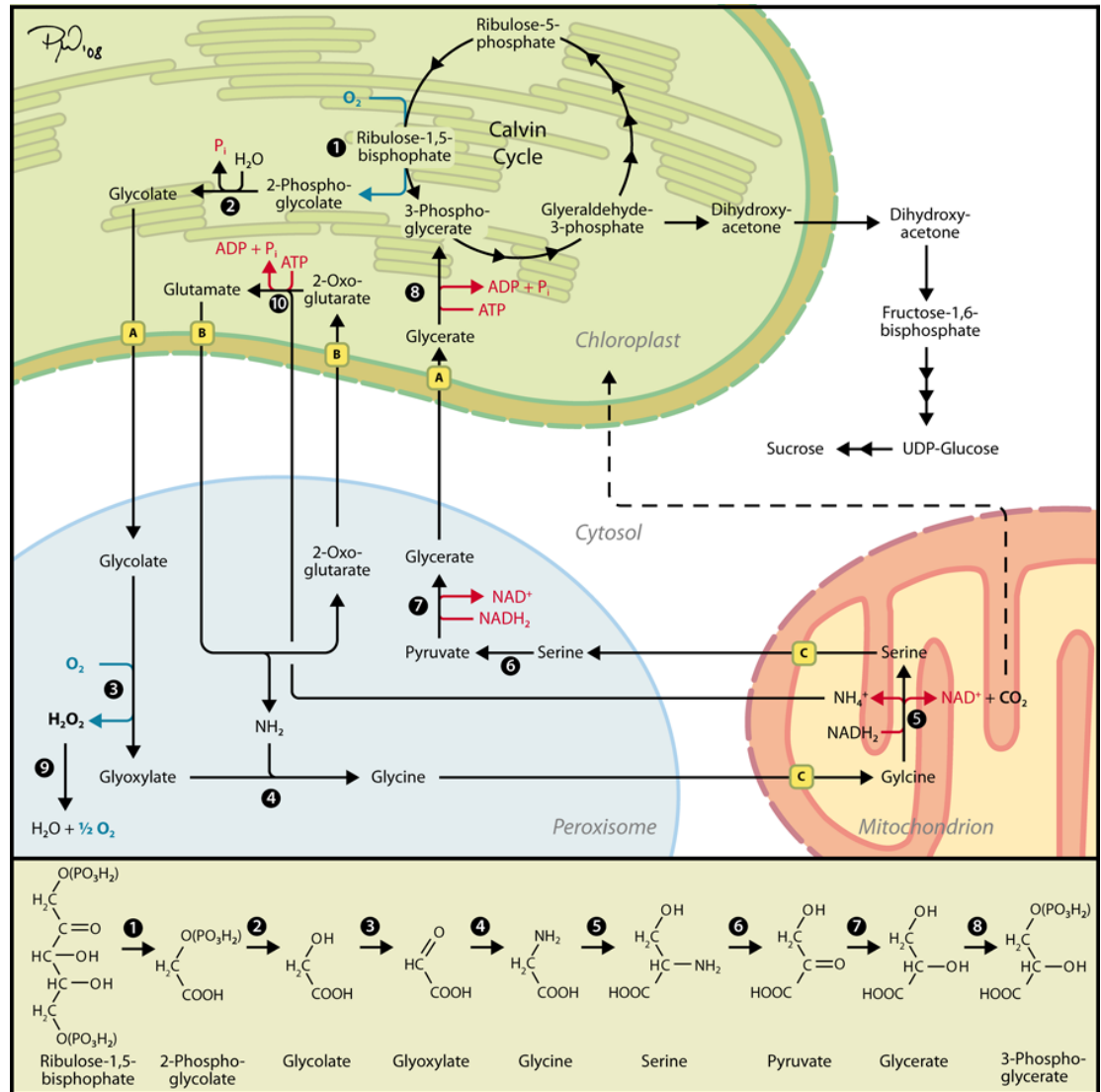
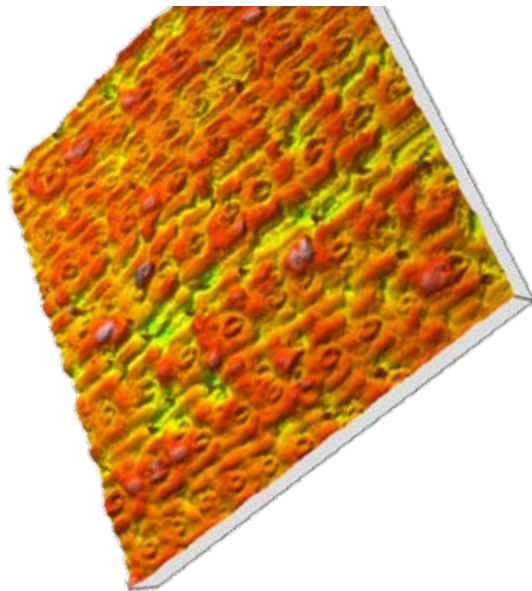
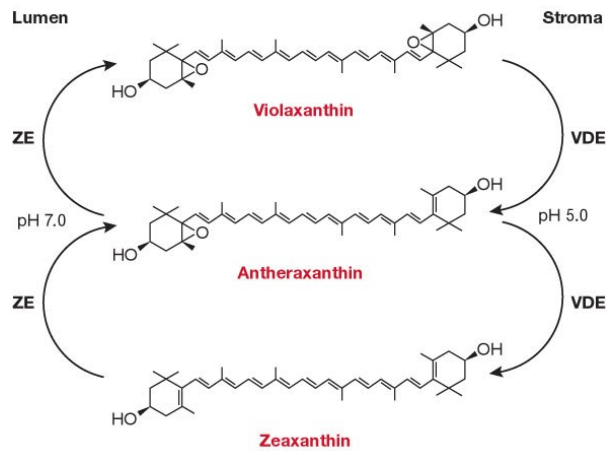


$$Y = 0.487 \cdot S_t \cdot \epsilon_i \cdot \epsilon_c \cdot \epsilon_p$$

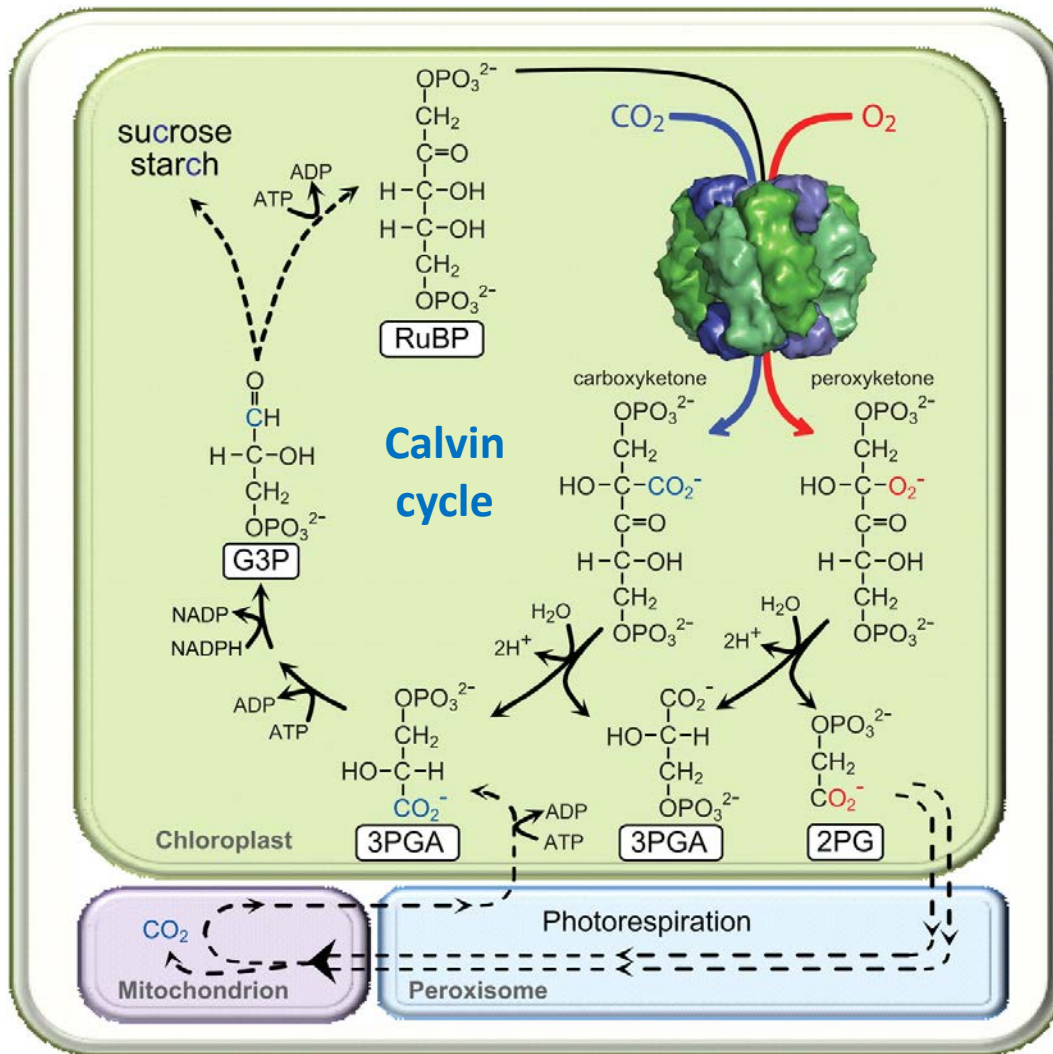




# Inefficiencies in photosynthesis

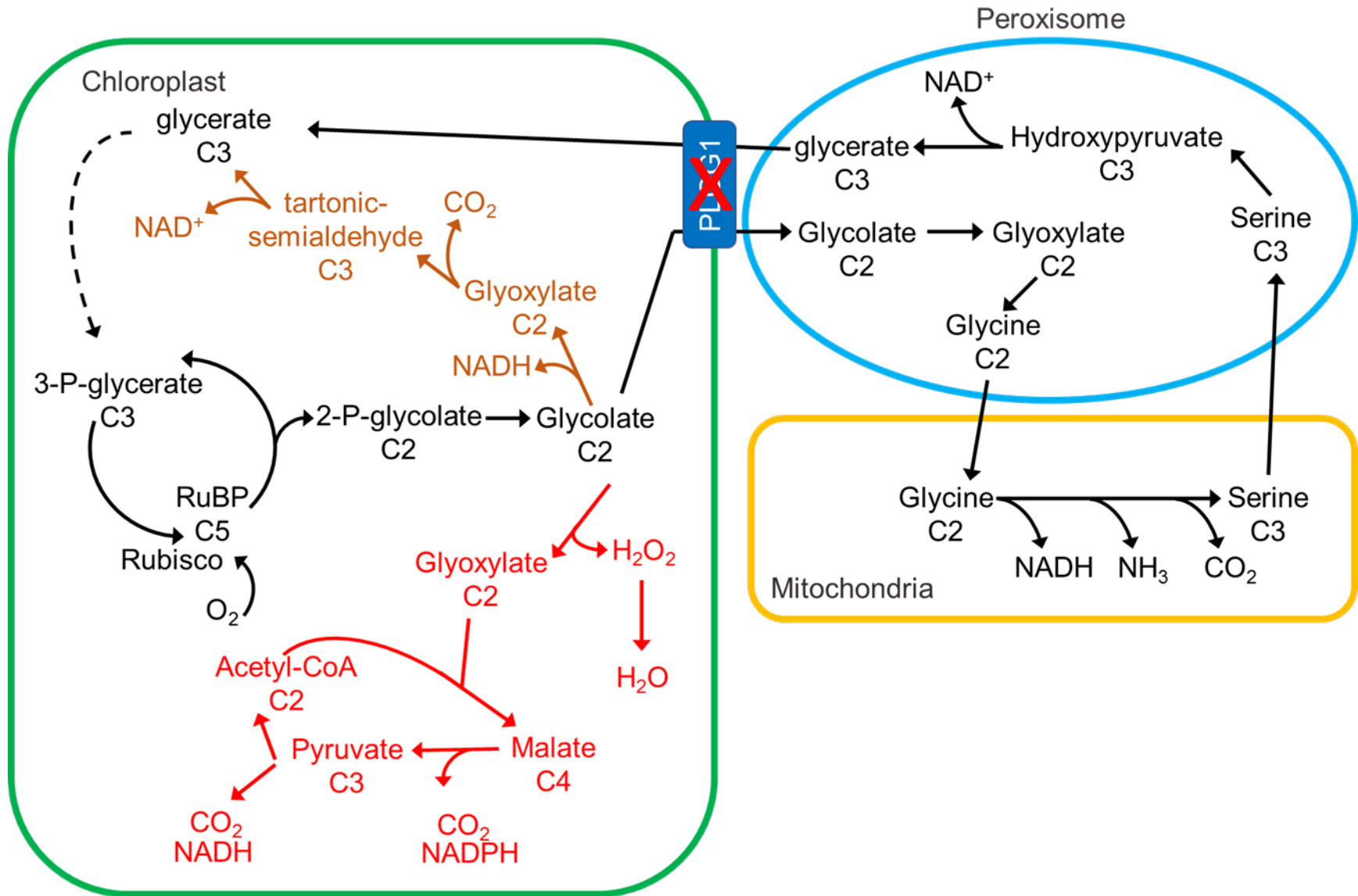


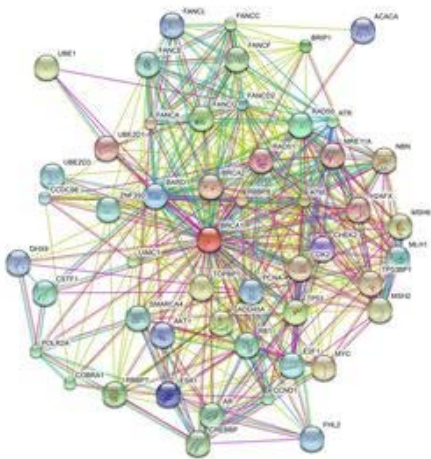
# Opportunity 1: Bypass Photorespiration



- 25% of Rubisco activity is oxygenation
- Recycling the toxic compound 2-phosphoglycolate (through photorespiration) is energetically expensive
- Estimated that photorespiration decreases soybean and wheat yields by ~148 trillion calories per year

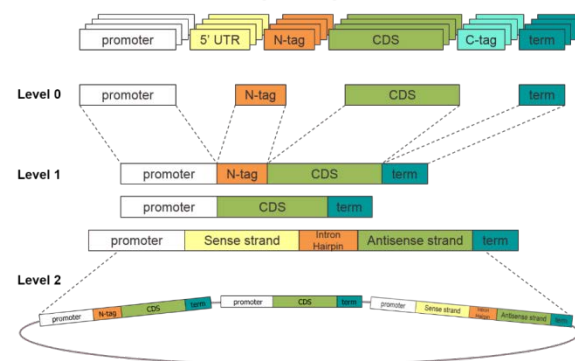
# Opportunity 1: Bypass Photorespiration





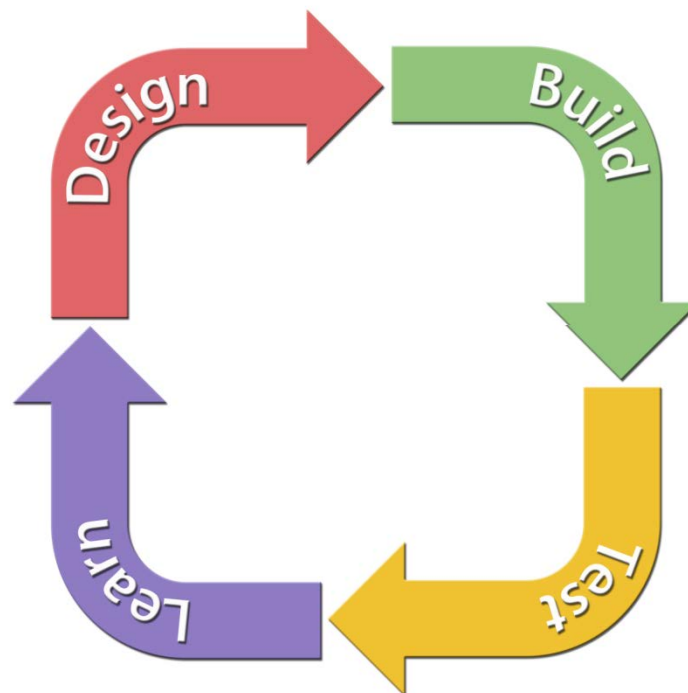
## Computation and Systems biology

Golden Gate allows modular cloning of multi-gene constructs



## Golden Gate Cloning DNA synthesis

## Plant transformation







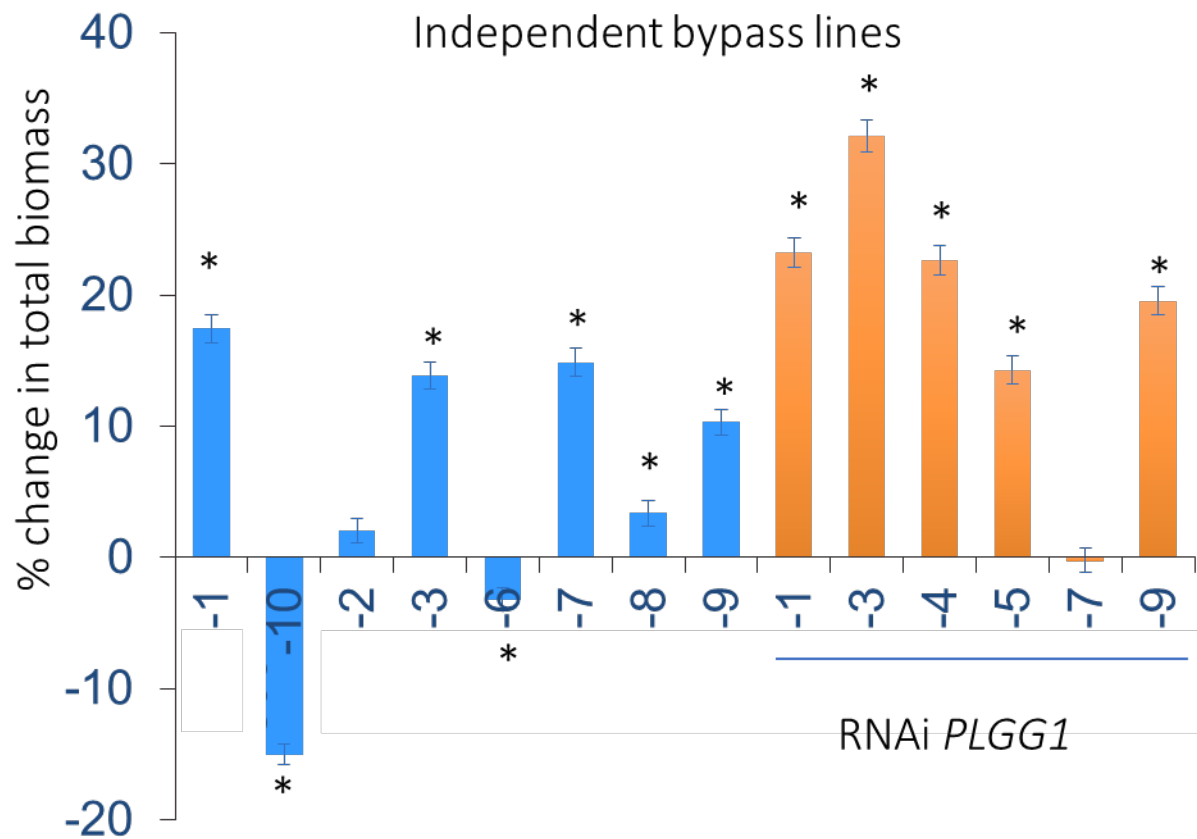
- 24 different photorespiration bypass designs
- With and without RNAi targeting PLGG1
- 140 single insert homozygous T2 lines have been confirmed from ~1500 segregating lines.

Photo taken at 8 weeks



WT

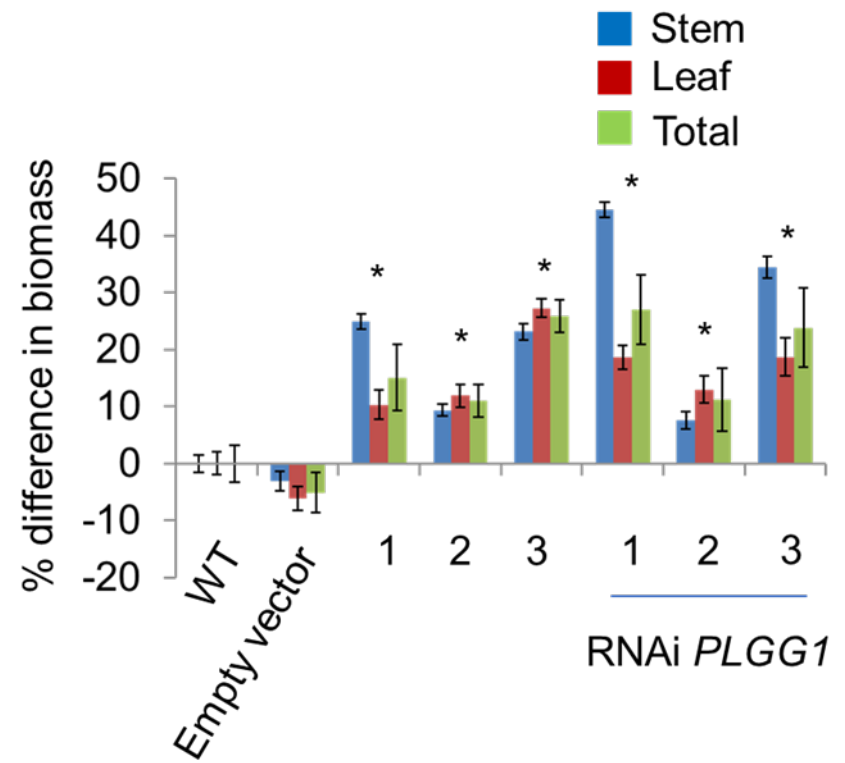
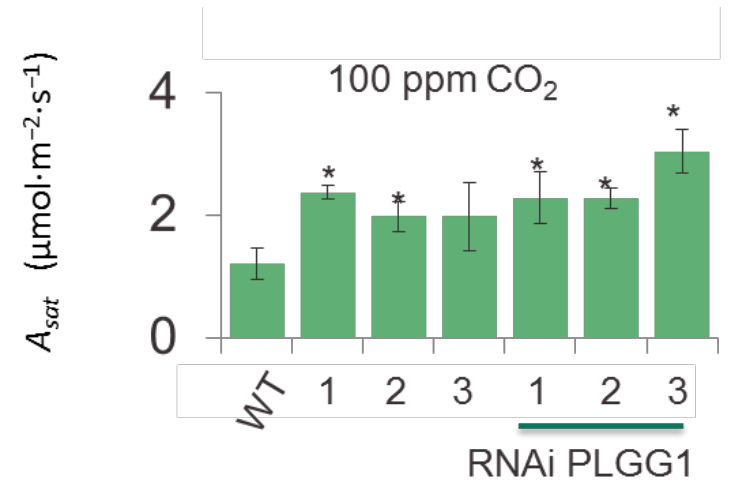
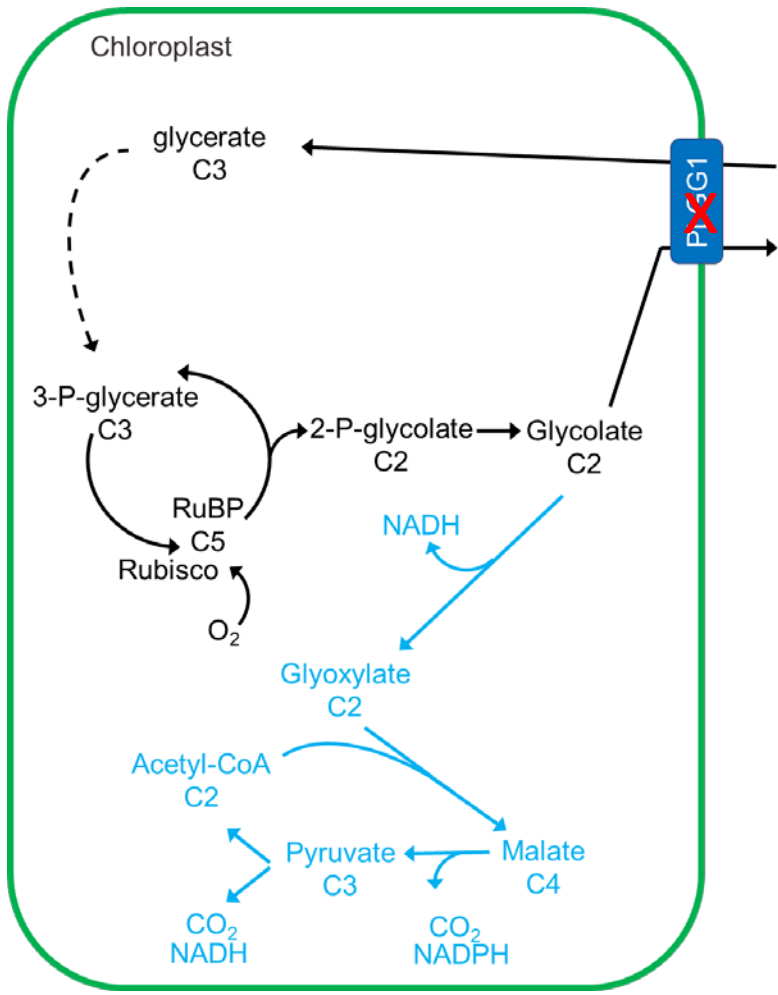
Bypass plus  
*PLGG1* RNAi



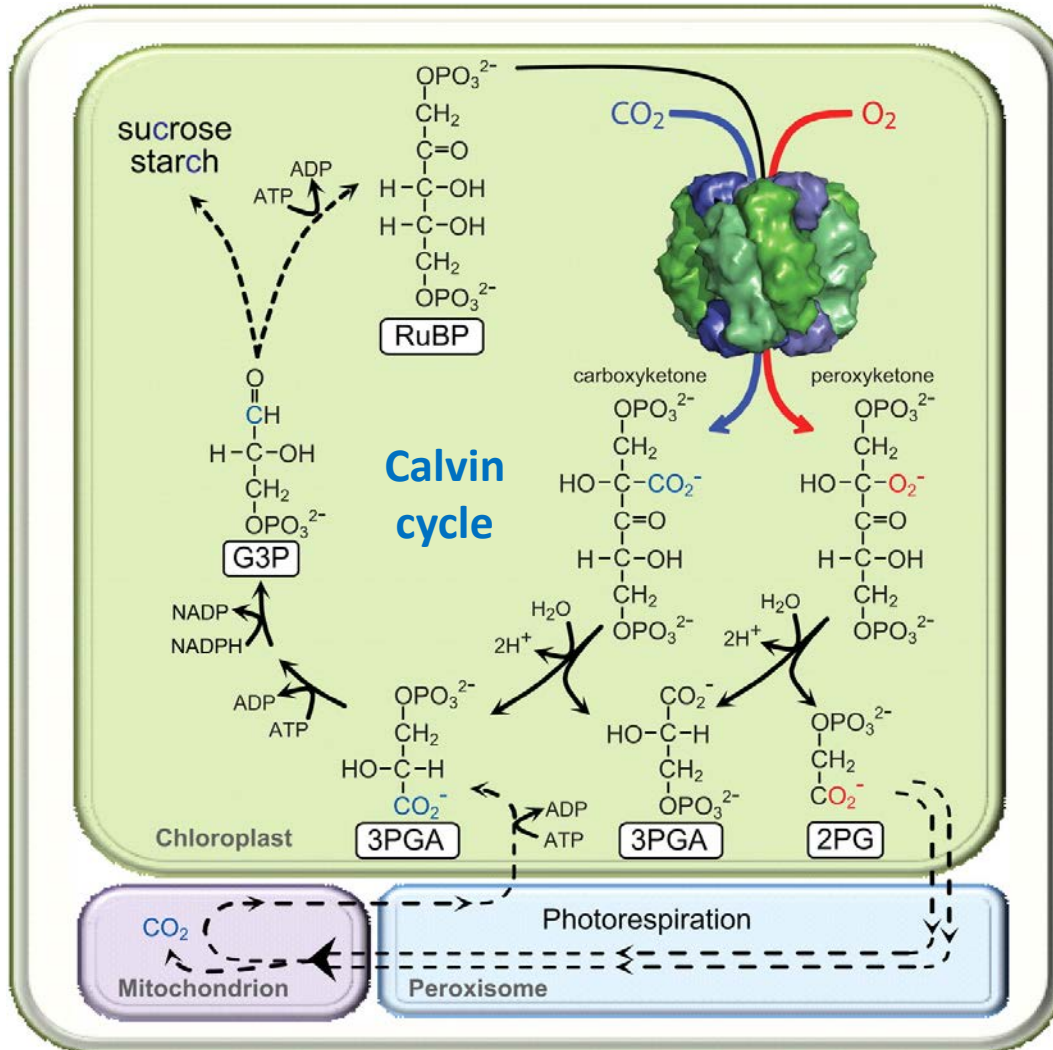
# Field Testing Promising Transgenic Lines



Paul South, Don Ort, et al.

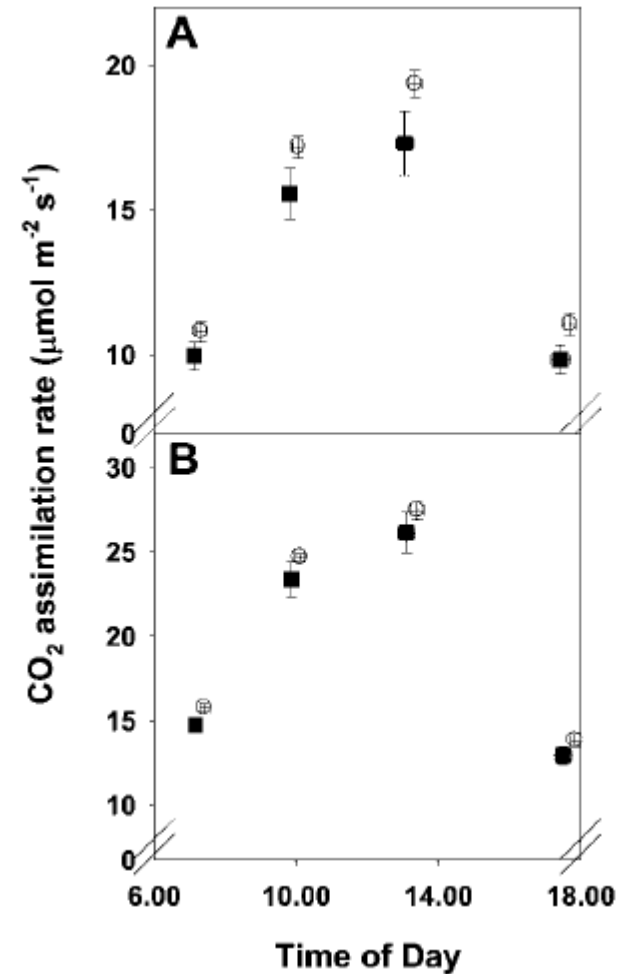
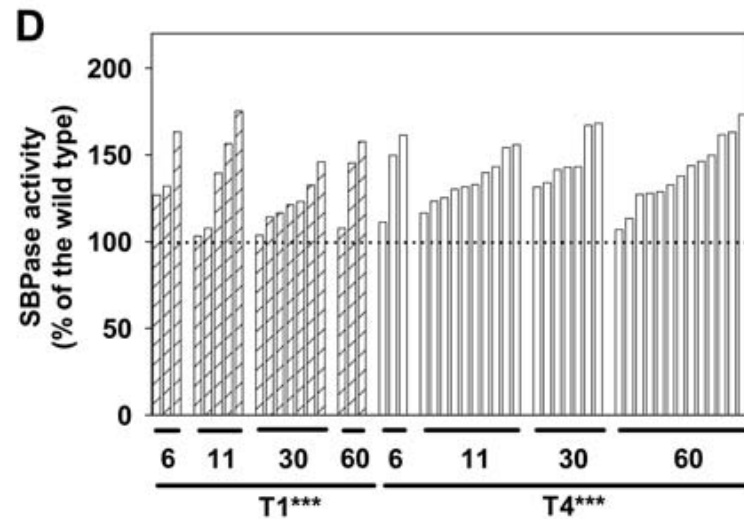
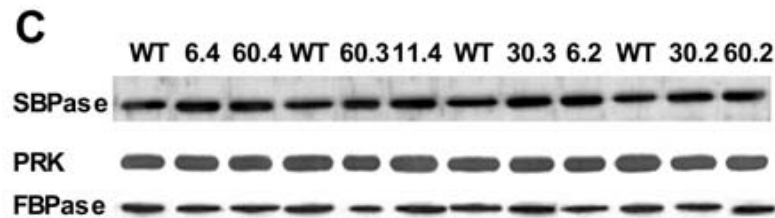
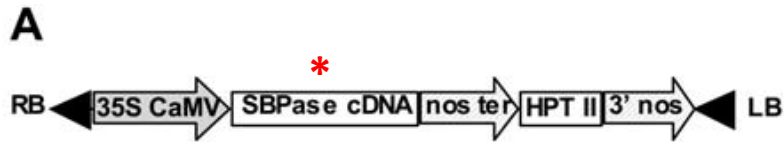


# Opportunity 2: Accelerate RuBP Regeneration



- RuBP is the sugar acceptor for  $\text{CO}_2$
- Eight enzymes are involved in the regeneration of RuBP for Rubisco
- Metabolic flux measurements and modeling suggest some of those eight enzymes have a role in determining rates of carbon flux into leaves

# Opportunity 2: Accelerate RuBP Regeneration



# Opportunity 2: Accelerate RuBP Regeneration



WT4

WT12

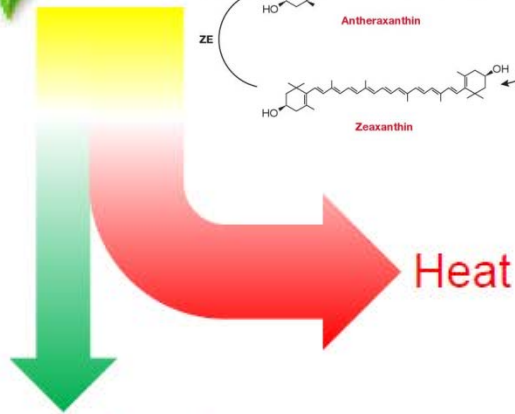
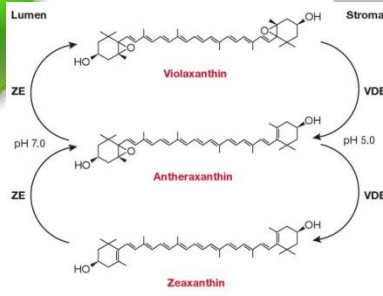
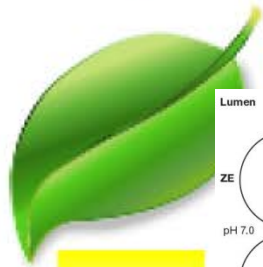
WT14

11.5

60.12

30.15

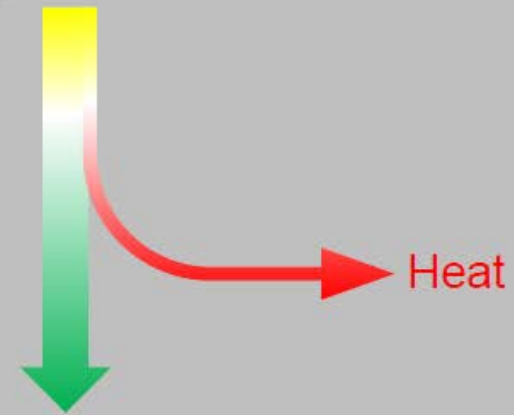
# Opportunity 3: Accelerate NPQ Relaxation



Photosynthesis



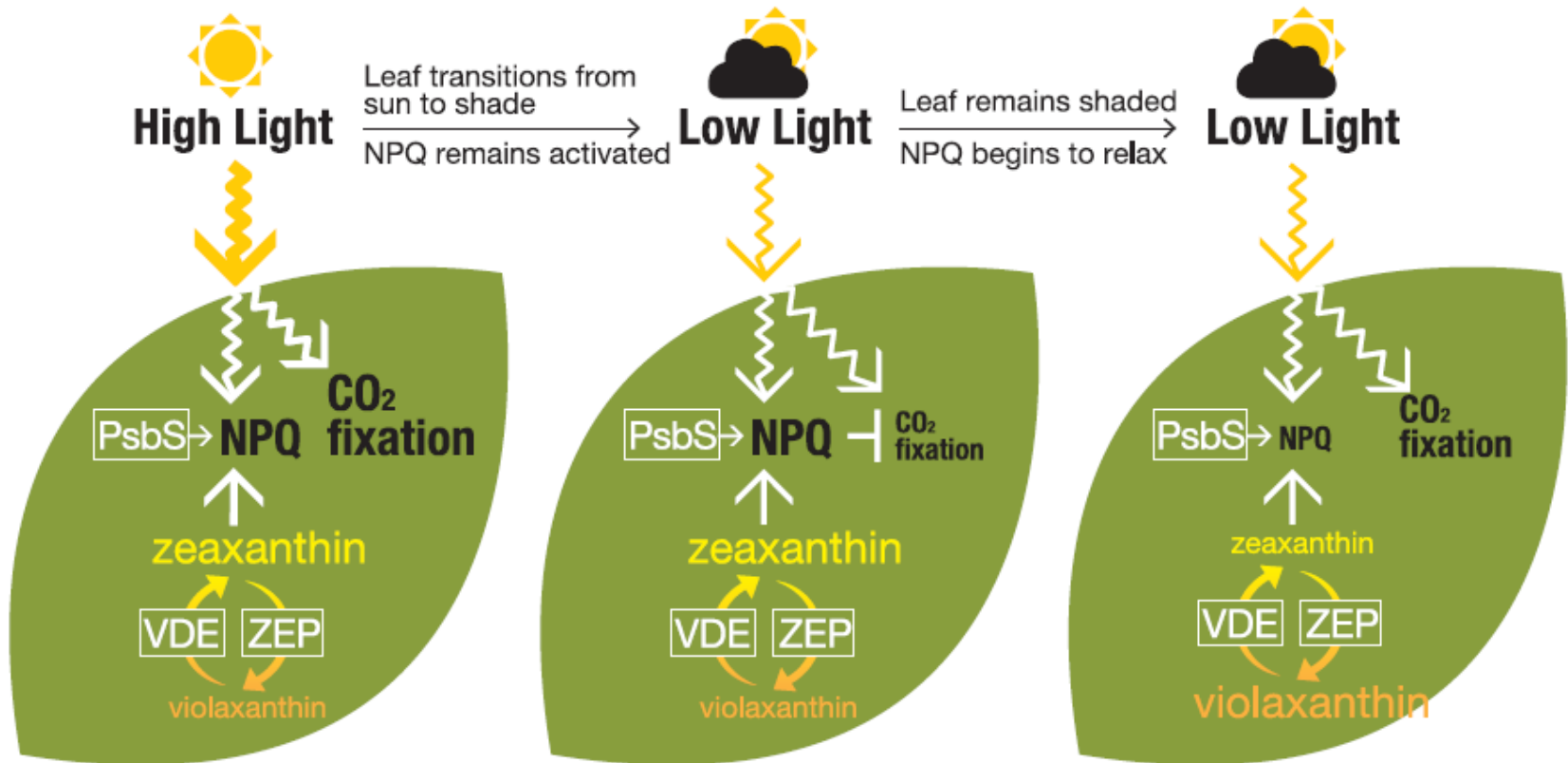
Photosynthesis



Photosynthesis



# Opportunity 3: Accelerate NPQ Relaxation



ZEP speeds up NPQ relaxation  
VDE balances ZEP activity during NPQ induction  
PsbS adjusts NPQ level to maintain WT amplitude

# Opportunity 3: Accelerate NPQ Relaxation

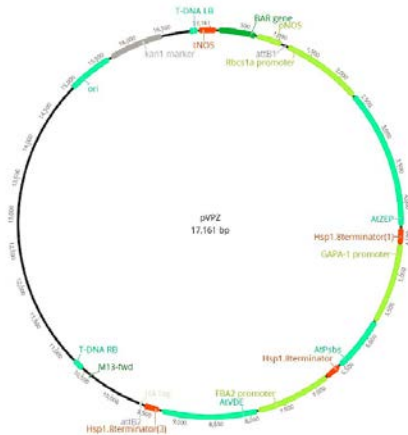
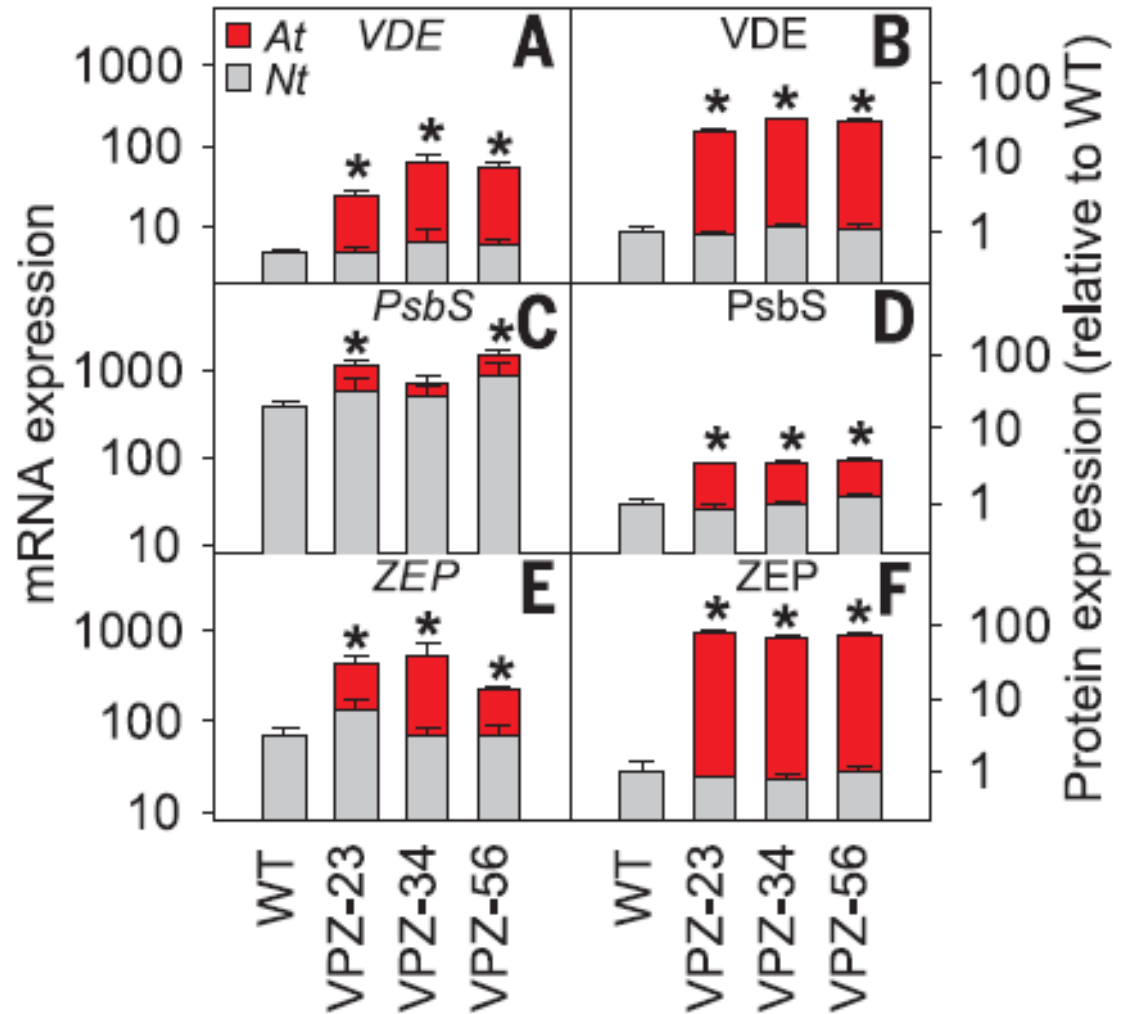


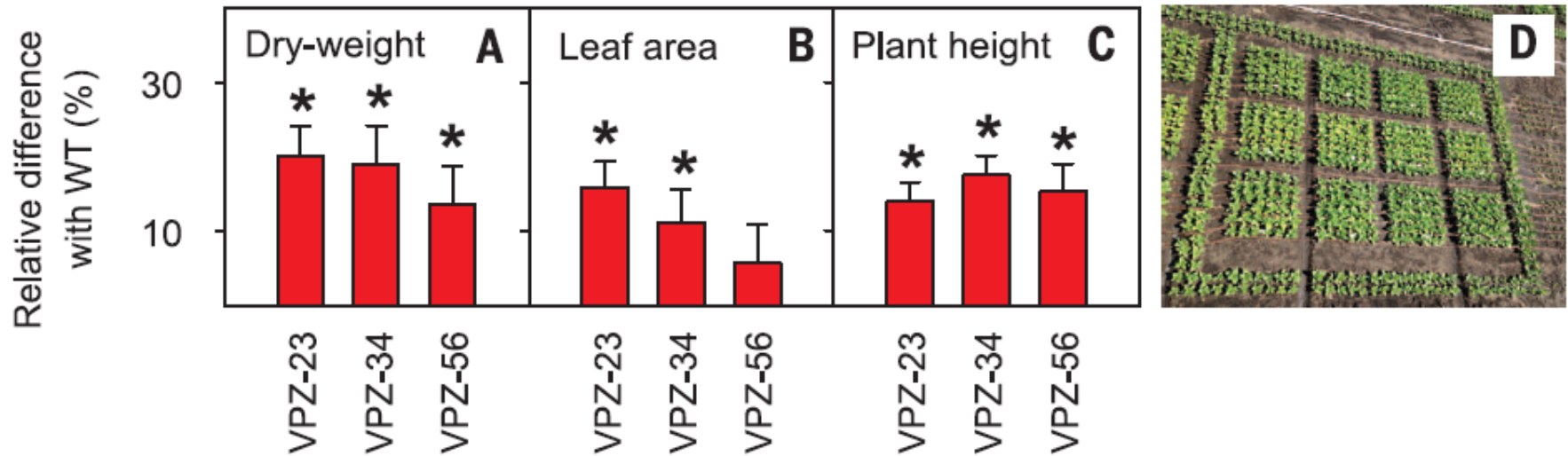
Fig. S1. Plasmid map of VPZ construct used to transform *N. tabacum*.



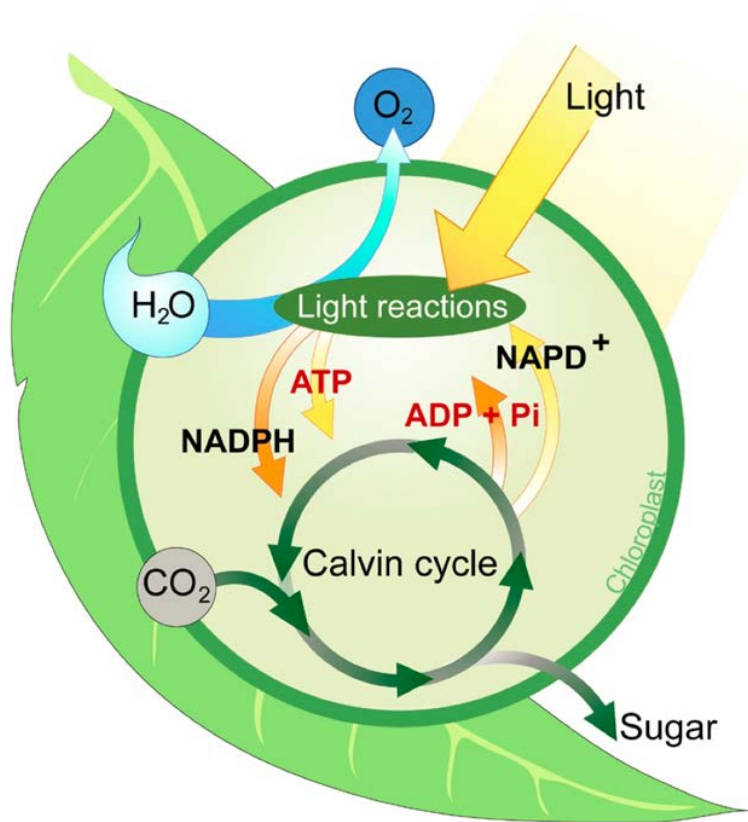
# Opportunity 3: Accelerate NPQ Relaxation



# Opportunity 3: Accelerate NPQ Relaxation



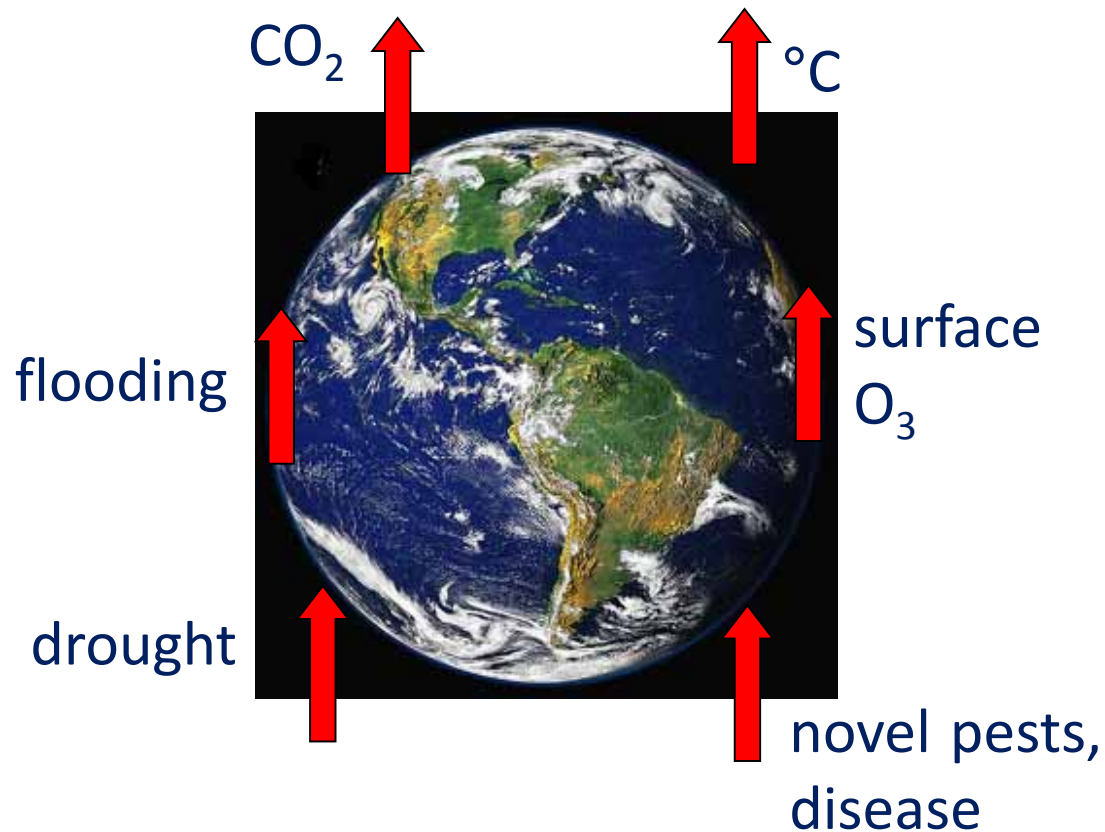
# Multiple opportunities to improve crop photosynthesis using transgenic strategies



**More efficient light utilization**  
(NPQ relaxation, altered canopy structure, more cytochrome b6f)

**More efficient carbon fixation**  
(Photorespiratory bypass, increased SPBase, altered Rubisco, activase)

# The growing environment is changing

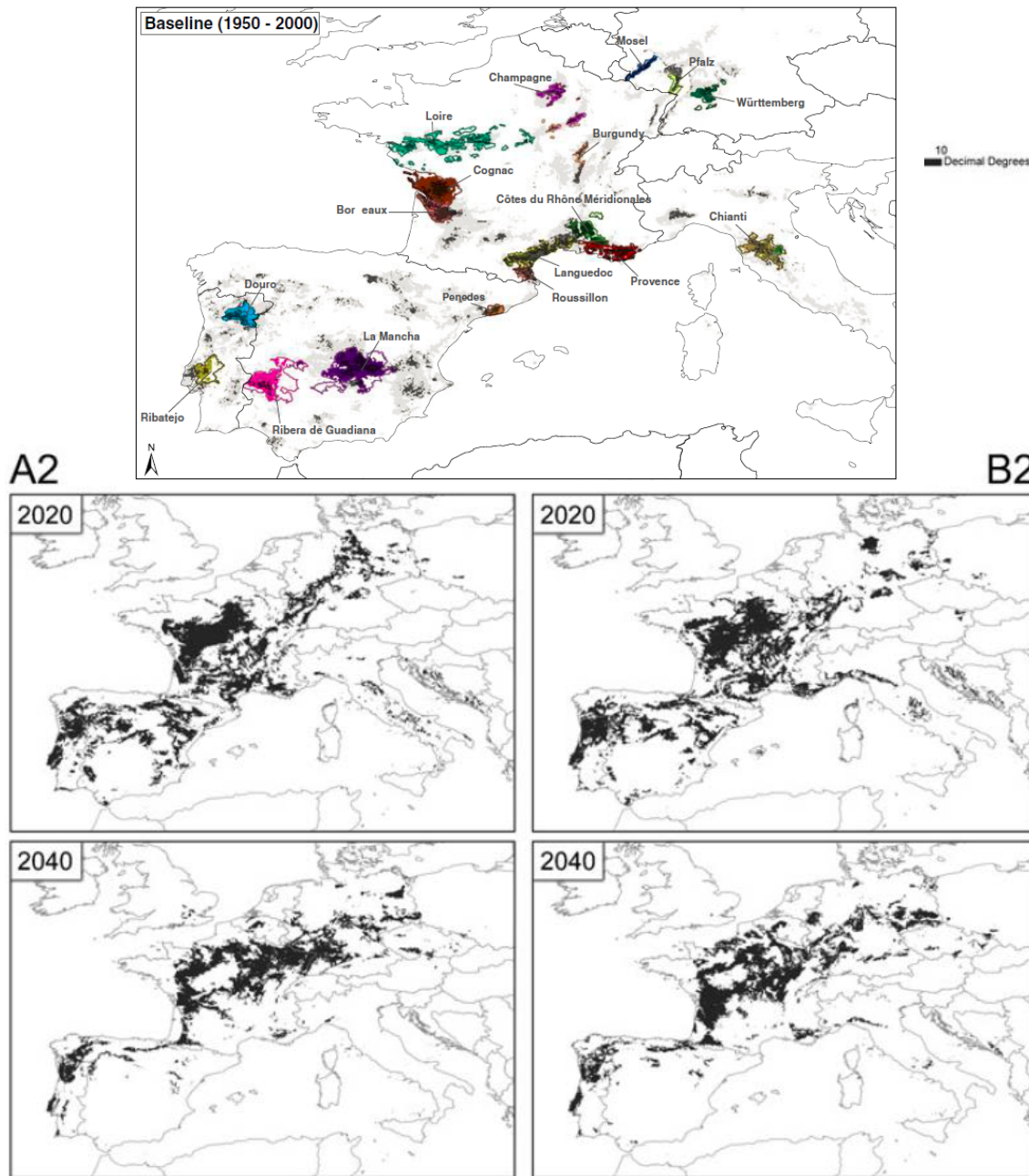


Adapting crops to a future growing environment, with increased  $[\text{CO}_2]$ , higher temperatures, altered water availability and increased pollution is a major challenge for agriculture.

# Climate Change Ripens Prospects For German Winemakers



<https://www.npr.org/sections/thesalt/2017/11/17/564099490/climate-change-ripens-prospects-for-german-winemakers>



# Climate Change Removes Prospects for Spanish Winemakers

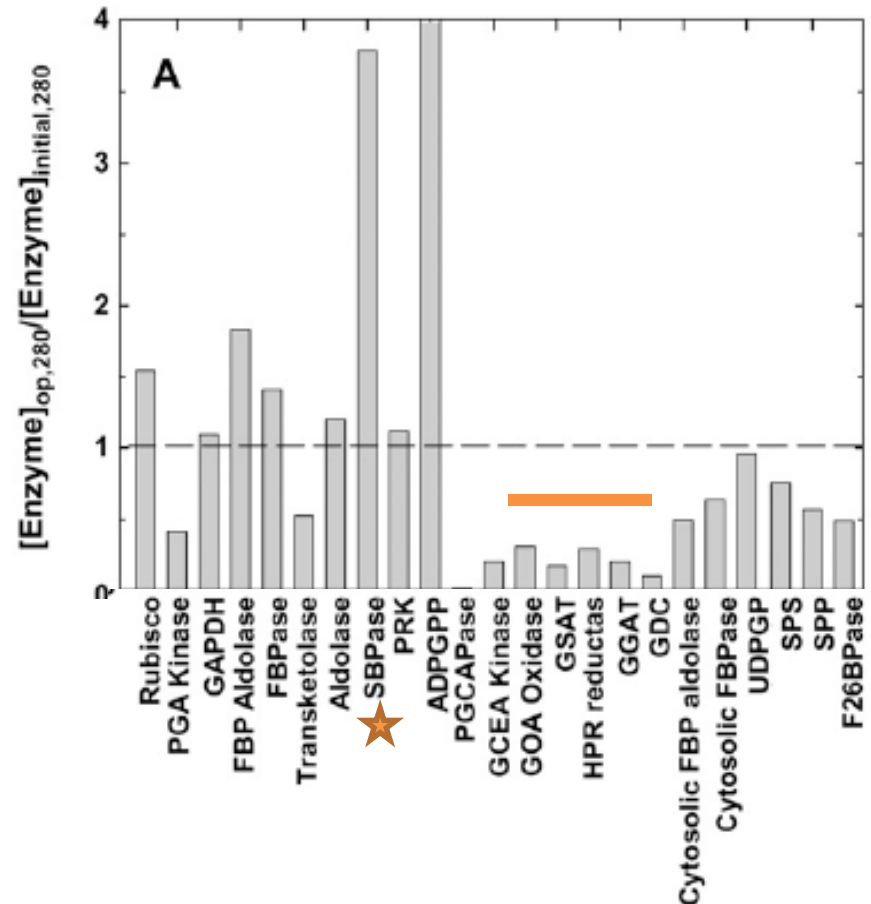
Fig. 2 Predicted grapevine cultivated area for the baseline (1950–2000) and the future time slices 2020 and 2050 in A2 and B2 scenarios



# Transgenic targets for improving crop responses to future climate conditions

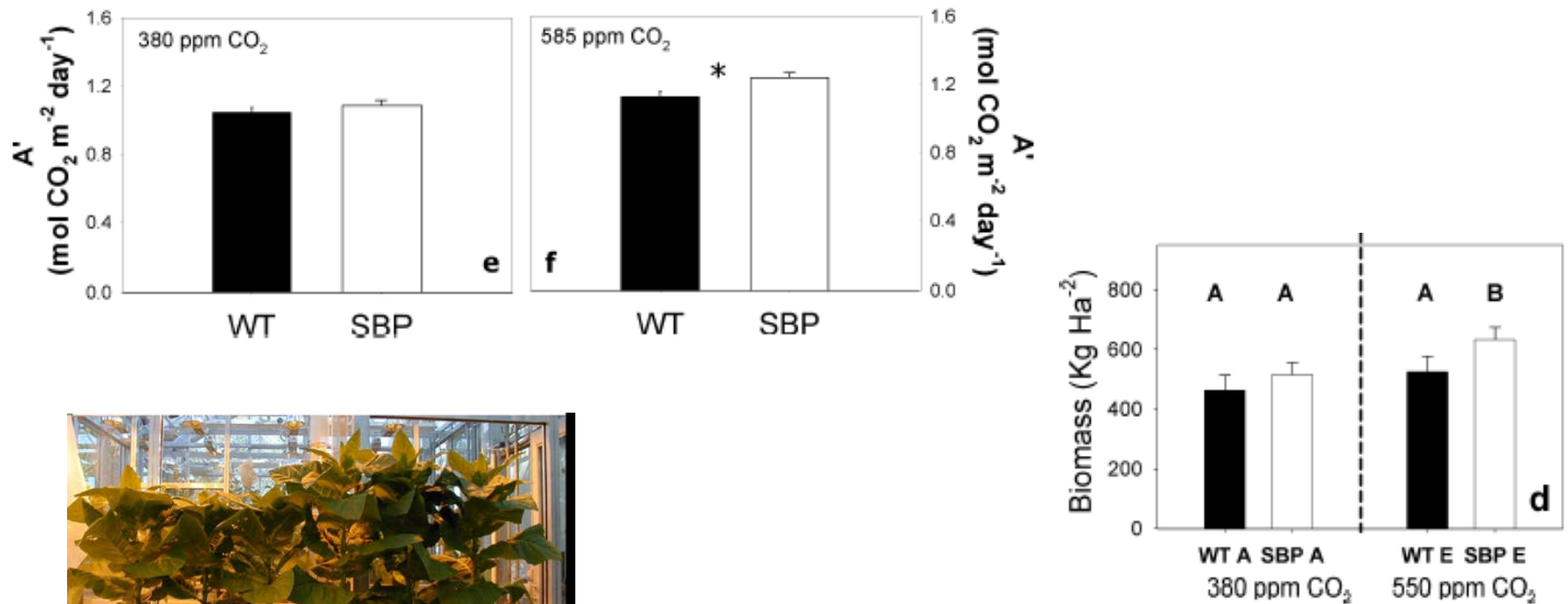
- Current partitioning of nitrogen among the enzymes of C<sub>3</sub> carbon metabolism is not optimized to today's atmospheric [CO<sub>2</sub>].

- Investment in photorespiratory enzymes is too high, while investment in SBPase, ADP-Glc pyrophosphorylase is too low.

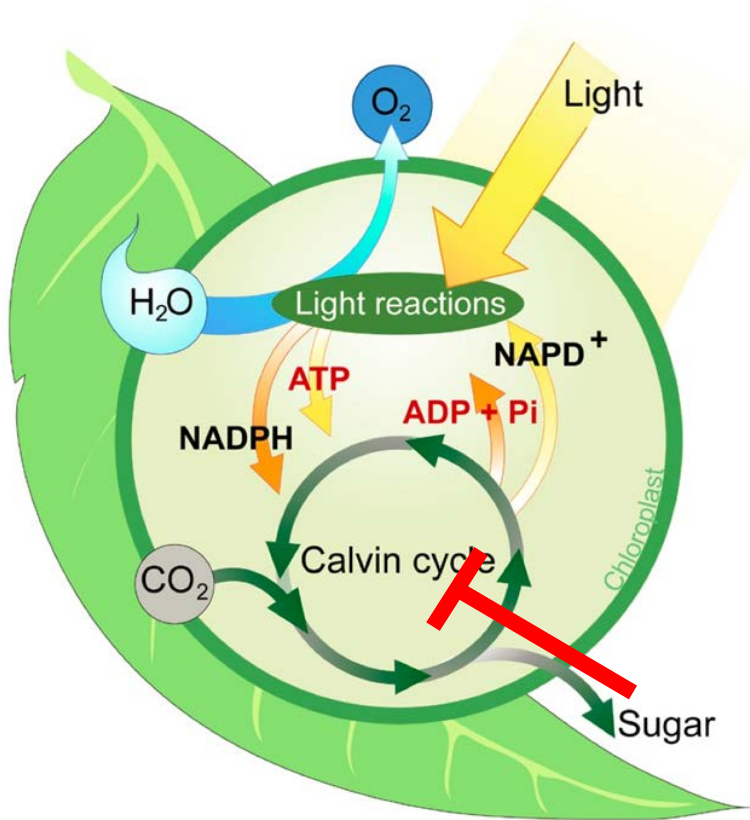


# Over-expressing the C<sub>3</sub> photosynthesis cycle enzyme Sedoheptulose-1-7 Bisphosphatase improves photosynthetic carbon gain and yield under fully open air CO<sub>2</sub> fumigation (FACE)

David M Rosenthal<sup>1</sup>, Anna M Locke<sup>2</sup>, Mahdi Khozaei<sup>3</sup>, Christine A Raines<sup>4</sup>, Stephen P Long<sup>5</sup> and Donald R Ort<sup>6\*</sup>

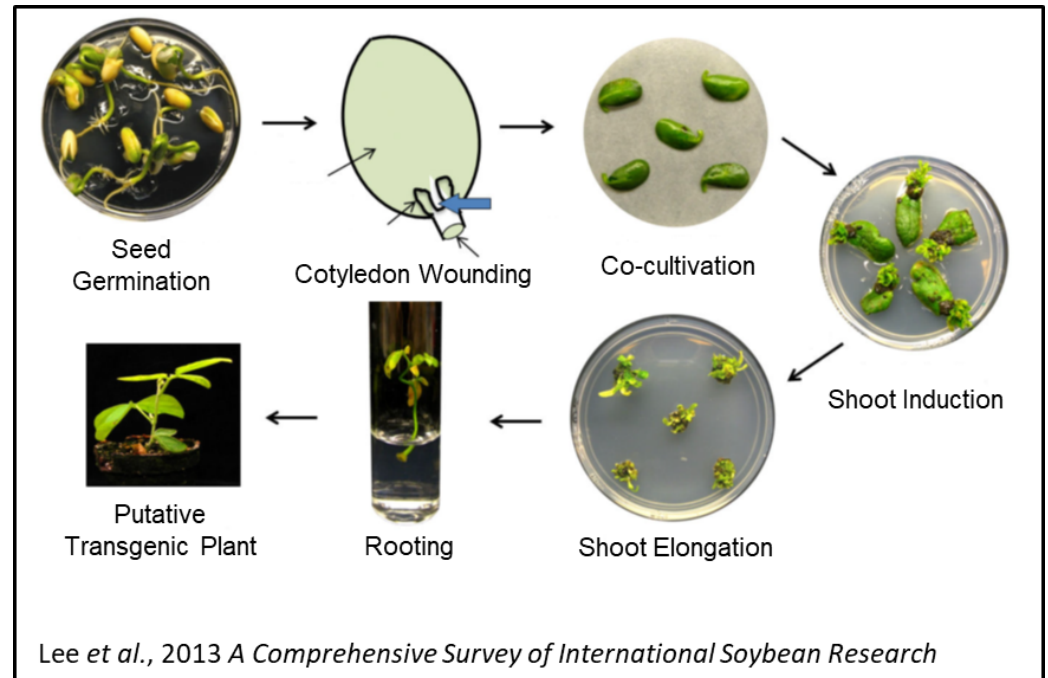
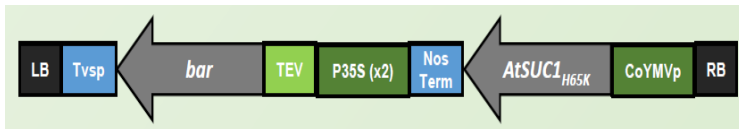


# Elevated $[CO_2]$ increases photosynthetic rate in C3 crops



**Increased sucrose concentration in leaves can negatively feedback on photosynthesis**

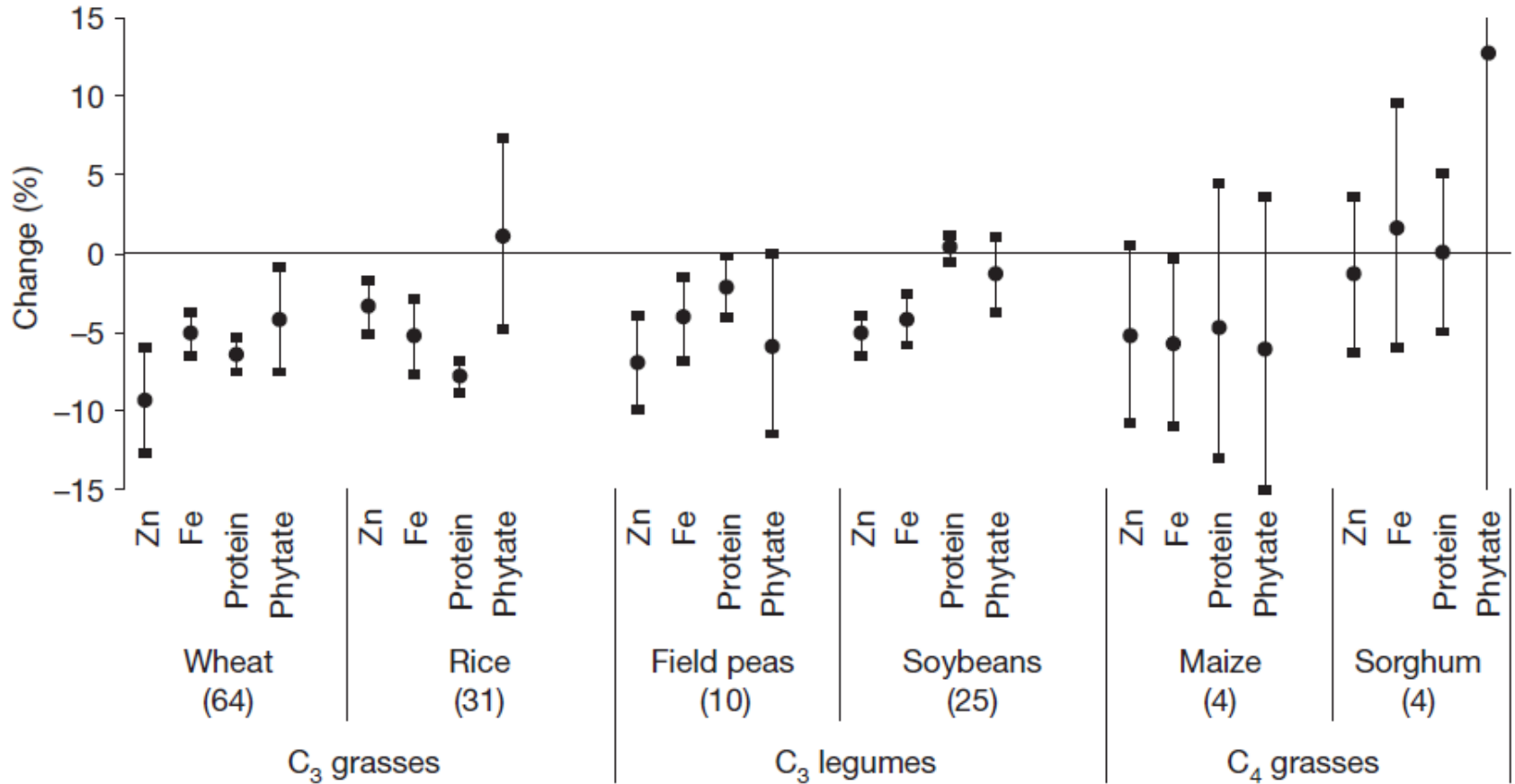
# Overexpress sucrose transporters



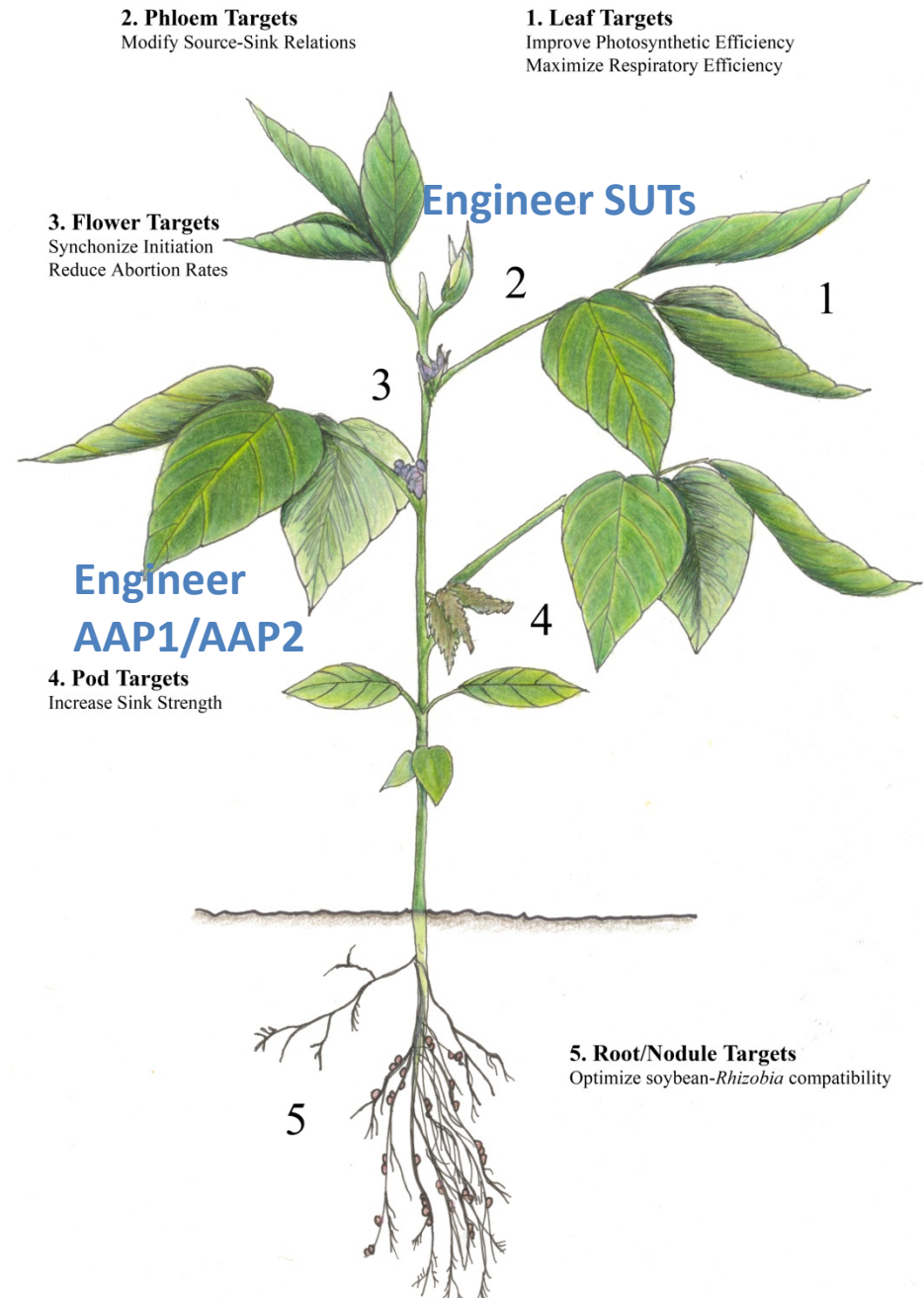
- **Gene of interest *AtSUC1*<sub>H65K</sub>**
  - Sucrose/proton symporter from *A. thaliana* with amino acid substitution
  - 14-fold higher transport activity than the native protein
- **Promoter from Commelina Yellow Mottle Virus**
  - Specific to the companion cells
  - Induced by sucrose

# Increasing CO<sub>2</sub> threatens human nutrition

Samuel S. Myers<sup>1,2</sup>, Antonella Zanobetti<sup>1</sup>, Itai Kloog<sup>3</sup>, Peter Huybers<sup>4</sup>, Andrew D. B. Leakey<sup>5</sup>, Arnold J. Bloom<sup>6</sup>, Eli Carlisle<sup>6</sup>, Lee H. Dietterich<sup>7</sup>, Glenn Fitzgerald<sup>8</sup>, Toshihiro Hasegawa<sup>9</sup>, N. Michele Holbrook<sup>10</sup>, Randall L. Nelson<sup>11</sup>, Michael J. Ottman<sup>12</sup>, Victor Raboy<sup>13</sup>, Hidemitsu Sakai<sup>9</sup>, Karla A. Sartor<sup>14</sup>, Joel Schwartz<sup>1</sup>, Saman Seneweera<sup>15</sup>, Michael Tausz<sup>16</sup> & Yasuhiro Usui<sup>9</sup>



Increasing the capacity for transporting sugars and amino acids to sinks and maximizing sink strength is also important – and will be increasingly as [CO<sub>2</sub>] rises.



# Summary

- Scientific and technological advancements enable rapid tests of potential targets to improve photosynthetic efficiency in crops
- As the climate changes, some growing regions may benefit, while others will suffer.
- New transgenic targets will be needed to adapt photosynthesis to global climate change.

# Acknowledgements

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Jennifer Quebedeaux

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Dan Bush



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