

# The effects of elevated [CO<sub>2</sub>] on soybean gene expression: An analysis of growing and mature leaves



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## INTRODUCTION

- Changes in leaf area index at elevated carbon dioxide concentration ([CO<sub>2</sub>]) drive changes in canopy carbon and water exchange.
- Larger individual leaf size is an important component of increased canopy leaf area index in plants grown at elevated [CO<sub>2</sub>].
- The molecular drivers of changes in leaf area are unknown, so we used soybean cDNA microarrays to investigate transcript changes in both rapidly growing and fully expanded leaves exposed to elevated [CO<sub>2</sub>] in fully open air conditions.

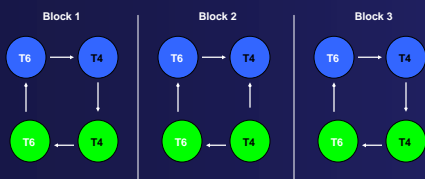
## METHODS

- Soybeans (*Glycine max* Merr. cv Pioneer 93B15) were grown at the SoyFACE facility, Urbana, Illinois ([www.soyface.uiuc.edu](http://www.soyface.uiuc.edu)) under ambient and elevated [CO<sub>2</sub>] (~375 and 550 ppm).



- Maximum growth rate in soybeans occurs during the middle of the night; therefore, samples were taken between 1 and 2 a.m. for transcript and metabolite analysis.

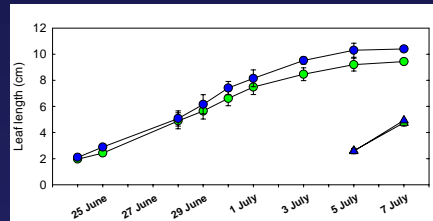
- Transcript profiles were analyzed with cDNA microarrays. Two libraries, Gm-1070 and Gm-1088 were probed, and over 18,000 genes were assessed. Two biological replicates, each containing 6 pooled leaflets, and two technical replicates were performed for each block of the experiment (n=3). **Figure 1** shows the loop design that was used to compare gene expression in **ambient** and **elevated** [CO<sub>2</sub>] and growing (**Trifoliolate 6**) and fully expanded (**Trifoliolate 4**) leaflets.



**Figure 1.** Design of the microarray experiment. Four "experiments" within each experimental block were compared. The first compared mature, 4<sup>th</sup> trifoliolate leaflets (T4) growing under ambient or elevated [CO<sub>2</sub>]. The second compared growing, 6<sup>th</sup> trifoliolate leaflets (T6) growing under ambient or elevated [CO<sub>2</sub>]. The third compared growing and fully expanded leaflets under ambient [CO<sub>2</sub>] and the fourth compared growing and fully expanded leaflets under elevated [CO<sub>2</sub>].

## RESULTS

- Elevated [CO<sub>2</sub>] increased leaf area (**Figure 2**), photosynthetic carbon uptake and daily carbon gain.

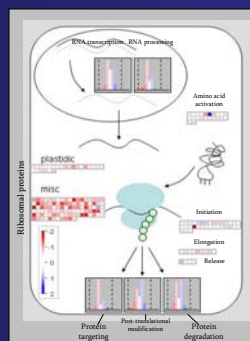


**Figure 2.** Increase in length of the fourth (circles) and sixth (triangles) trifoliolate lateral leaflets grown at ambient (green) and elevated (blue) [CO<sub>2</sub>]. Samples for microarray analysis and leaf carbohydrates were taken on July 8, 2004 between 1 and 2 AM, when T4 leaflets were fully expanded and T6 leaflets were expanding in length at 42 ± 6% d<sup>-1</sup>.

- While midday carbohydrate levels were significantly higher at elevated [CO<sub>2</sub>], midnight carbohydrate levels were not significantly affected by elevated [CO<sub>2</sub>], suggesting more rapid utilization of starch at elevated [CO<sub>2</sub>].

## GENES ASSOCIATED WITH LEAF DEVELOPMENT

- Expression of 1146 genes was significantly different in growing versus fully expanded leaves, irrespective of growth [CO<sub>2</sub>].
- 132 transcripts showed at least one and a half times higher expression in growing leaves, and therefore represent our best estimates of control points of leaf expansion. These included ribosomal proteins, cell cycle genes, histones, cell wall loosening genes and tubulin genes. Leaf expansion was likely due to cytoplasmic growth and cell proliferation, based on the abundance of highly expressed ribosomal proteins (**Figure 3**).

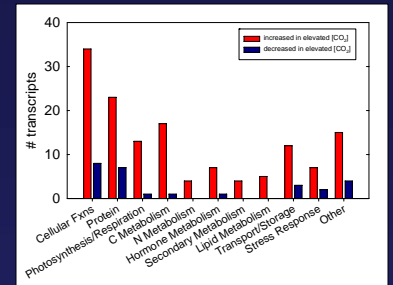


**Figure 3.** Developmental effects on transcripts involved in RNA processing and protein synthesis. Red boxes denote transcripts that were up-regulated in developing leaflets and blue boxes denote down-regulated transcripts.

## [CO<sub>2</sub>] RESPONSE GENES

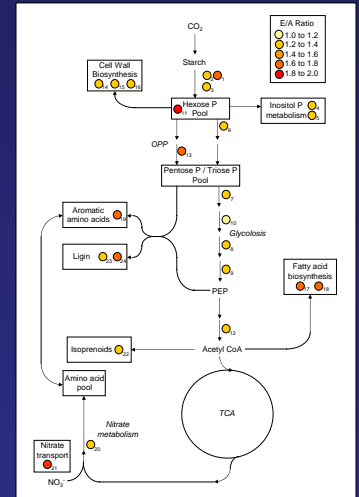
- 327 genes showed a significant response to elevated [CO<sub>2</sub>], and were assigned to different functional categories (**Figure 5**).

- Many genes with roles in cellular functions (i.e. cell cycle, RNA regulation of transcription, DNA synthesis and cell organization) showed higher expression in elevated [CO<sub>2</sub>]. Within this category, most of the genes with higher expression in elevated [CO<sub>2</sub>] were transcription factors.



**Figure 5.** Categorical distribution of genes showing differential expression under elevated [CO<sub>2</sub>].

- Many genes encoding enzymes of central carbon metabolism were up-regulated at elevated [CO<sub>2</sub>] (**Figure 6**).



**Figure 6.** Graphical representation of selected gene transcripts up regulated in response to growth at elevated [CO<sub>2</sub>]. The colored dots indicate that a gene encoding an enzyme for that step or pathway is significantly up regulated at elevated [CO<sub>2</sub>]. The color of the dot indicates the degree of up regulation at elevated [CO<sub>2</sub>] relative to ambient [CO<sub>2</sub>] controls (E/A ratio) the darker the color, the greater the up regulation.

- Beta-amylase
- Myo-inositol-1-phosphate synthase
- Inositol-3-phosphate synthase
- Phosphotransferase
- Glyceraldehyde 3-phosphate dehydrogenase
- Phosphoglycerate mutase
- Enolase
- Cytosolic phosphoglycerate kinase
- Plastidial phosphoglucomutase
- Putative pyruvate dehydrogenase
- Phosphogluconate dehydrogenase
- NAD dependent epimerase
- Cellulose synthase
- UDP-D-xylose synthase
- Palmitoylacyl carrier protein thioesterase
- Enoyl-ACP reductase
- Indole-3-glycerol phosphate synthase
- Glutamine synthase precursor
- Putative nitrate transporter
- 1-deoxy-D-xylulose 5-P reductoisomerase
- Caffeic acid methyl transferase
- Cinnamoyl-CoA reductase

## CONCLUSION

In this field study, we tested the hypothesis that increased carbon assimilation in plants grown at elevated [CO<sub>2</sub>] altered pools of carbohydrates and transcripts that control growth and expansion of young leaves. It is well established that elevated [CO<sub>2</sub>] increases photosynthetic carbon fixation and carbohydrate synthesis; however, this research shows that at the transcript level, elevated [CO<sub>2</sub>] also stimulates the respiratory breakdown of carbohydrates, which provides increased fuel for leaf expansion and growth at elevated [CO<sub>2</sub>].