Elevated CO_2 differentially affects gene expression and metabolite profiles in *Arabidopsis thaliana* ecotypes and in *Thellungiella halophila*

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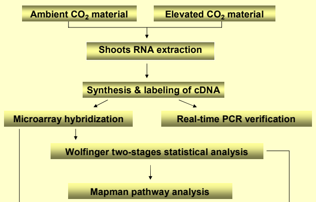
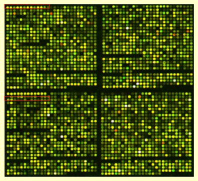
Introduction

Global atmospheric CO_2 concentration [CO_2] has increased to its highest level in recent history and is expected to nearly double during this century. Insight into how plants respond and adapt to the projected increase in [CO_2] is important, and understanding will also help to better comprehend the biochemistry of carbon assimilation in general and the consequences for plant performance in a shifting global scenario to which plants must adapt. The UIUC SoyFACE (Free-Air Concentration Enrichment) facility, which simulates atmospheric conditions expected by 2050, provides great advantage to gauge plant responses to elevated CO_2.

In this study, we grew Arabidopsis *thaliana* Col-0, Cvi-0, WS and *Thellungiella halophila* in the field within SoyFACE, harvested them after a 10-14d exposure to elevated CO_2, used Arabidopsis long oligo microarrays combined with metabolite profiles by GC-MS to monitor effects of elevated CO_2 on transcript and metabolite abundance. The results indicated that a core set of signature processes and genes reporting atmospheric CO2 response pathways united *Thellungiella* and the three *Arabidopsis* ecotypes, while different behavior in several metabolic pathways distinguished ecotypes among each other and with respect to Thellungiella.

Experiment and analysis flow chart





Segments from the *Arabidopsis* oligo-array slide

(total 26,000 elements, control elements are boxed)

Wolfinger two-stages statistical analysis

First – Normalization Model Fitting

$$y_{gijklm} = \mu + T_i + D_j + A_k + (T\times A)_{ik} + B_{l(k)} + \varepsilon_{gijklm}$$

y: Log intensity values μ: Overall means T: Treatment D: Dye

A: Array T×A:Treat-array interaction B: Block within array

Second - Gene Model Fitting

$$\varepsilon_{gijklm} = G_g + (G\times T)_{gi} + (G\times D)_{gj} + (G\times A)_{gk} + (G\times S)_{gm} + \lambda_{gijklm}$$

ε: Residuals from normalization G: Gene specific mean λ: Residuals

G×T: Gene treatment interaction G×D: Gene Dye interaction

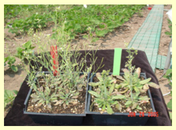
G×A: Gene array interaction G×S: Gene spot interaction

Assessment of significantly differential expressed genes

Results

Cvi-0 grew best in elevated CO_2 (2003)

In June 2003, three Arabidopsis ecotypes (Col-0, Cvi-0, WS) and a related species, Thellungiella (Th), were grown in elevated [CO_2] at 550 ppm in the field within the UIUC SoyFACE facility for 12 days. Cvi-0 and Th grew relatively slowly, exhibiting green and healthy leaves at the time of harvest, while, in both ambient and elevated CO_2, WS had begun to flower, and Col-0 showed siliques with developing seeds after 10 days.



Col-0 (left) and Cvi-0 (right) grew in elevated CO_2 (2005 Jun)

Table 1. Photosynthesis parameters (2005)

Ring	Ecotype	A	g_s	Ci
Ambient CO_2	Col-0	6.62 ± 0.93	0.20 ± 0.02	319.75 ± 12.86
	Cvi-0	11.07 ± 2.18	0.26 ± 0.10	297.05 ± 12.81
Elevated CO_2	Col-0	11.66 ± 0.68	0.14 ± 0.02	392.83 ± 26.42
	Cvi-0	20.74 ± 2.16	0.31 ± 0.07	394.35 ± 52.34

A: Light saturated CO2 assimilation

g_s: Stomata CO_2 conductance Ci: Intercellular [CO_2]

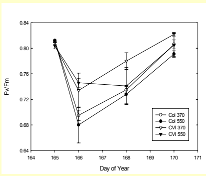


Fig. 1 The maximum quantum yield of PSII (Fv/Fm), measured in *Arabidopsis* genotypes Columbia (Col-0) and Cape Verde Island (Cvi-0).

370 ppm - ambient [CO_2]

550 ppm - elevated [CO_2]

Repeat experiment in June 2005 (Col-0 and Cvi-0).

Gas exchange was measured in fully expanded rosette leaves after plants had been exposed to elevated [CO_2] in the field for 6 days. There was significant variation between the cultivars; Cvi-0 had higher rates of photosynthesis (*A*) than Col (Table 2). There was also a significant stimulation of *A* in elevated [CO_2]. Rates for both genotypes roughly doubled under elevated [CO_2].

The maximum quantum yield of PSII (Fv/Fm) was measured before plants were taken to the field (DOY 165) was ~0.80 for both cultivars (Fig. 1). Fv/Fm decreased upon initial exposure to field conditions (DOY 166), but recovered within 3 days in the field, suggesting that plants overcame the initial stress imposed by exposure to the field environment

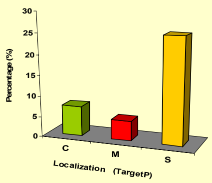
Table 2 Statistical results using the two-stages method by Wolfinger et al. (2003)

Comparison	Statistical results from Wolfinger				
Log_2 (Ambient CO_2 vs elevated CO_2)	Posit.	Negat.	No change	No value	Total
Col-0	818	481	21562	115	22976
Cvi-0	174	390	22296	62	22922
TH	1107	608	20500	32	22247
WS	859	372	21871	185	23287

In addition to controls, the microarray platform included ~25,000 probes. Only probes which passed the Wolfinger two-stage statistical analysis were accepted as significant differential expressed genes. Cvi-0 had the smallest and Th the largest number of up/down regulated genes in the four lines.

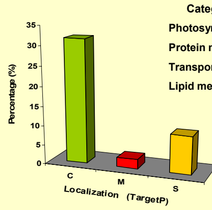
A common set of genes regulated in different lines ……

A Up-regulated genes



Category	Percentage (%)	Genes (examples)
N - metabolism	10.98	SDH, GDH2
Redox related	10.98	ATP38, SRG1
Signaling	10.98	FRY1, RALFL23
C – metabolism	9.76	GWD2, PEPCK
Transport	8.54	MIP, ABC transporter

B Down-regulated genes



Category	Percentage (%)	Genes (examples)
Photosynthesis	21.95	OEE, TMP14, GAP, CA1
Protein modification	9.76	PP2A, PP2C
Transport	9.76	Mt carrier, KEA1
Lipid metabolism	4.88	Lipase, MFP2

Fig.2 Localization prediction of genes regulated in the same direction in four lines .

A- up regulated genes

B- down regulated genes

C – chloroplast, M - mitochondria, S - secretory pathway

Different lines have different strategies for elevated CO_2 ……

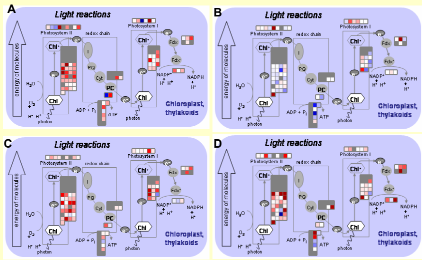


Fig 3. Light reaction response to the elevated CO_2

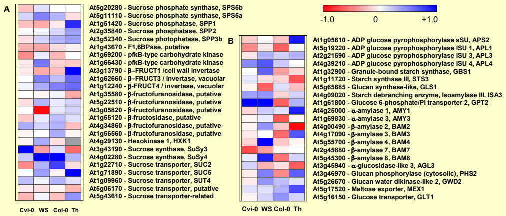
A - Cvi-0, B - WS, C - Col-0, D - Th

Grey squares denote chloroplast-encoded transcripts which were not included in the array and transcript hybridization signals that did not pass the normalization filters. Up-regulation is indicated by shades of blue, down-regulation by red (Mapman representation).

Table 3 Metabolites in elevated CO_2 (μg/g FW)

Metabolites	Cvi-A	Cvi-B	WS-A	WS-B	Col-A	Col-B	Th-A	Th-B
Organic acids	3964.1 ±130.1	2453.1 ±80.0	6476.4 ±115.9	7655.2 ±180.2	8791.3 ±160.3	7364.9 ±240.4	8723.1 ±231.1	7124.3 ±120.1
Amino acids	721.1 ±17.6	451.5 ±10.0	1619.7 ±322.6	2042.5 ±68.0	4572.0 ±55.8	2988.0 ±179.0	8189.9 ±321.0	7634.5 ±65.6
Hexoses and hexose – P	478.0 ± 5.0	159.8 ± 15.3	1567.3 ± 25.4	1813.6 ± 70.0	3289.9 ± 56.0	2779.8 ± 144.7	4400.5 ± 214.6	6142.7 ± 68.4
Disaccharides	2364.4 ± 66.5	2112.8 ± 35.5	3940.2 ± 216.6	4747.7 ± 294.1	4807.9 ± 168.8	4167.6 ± 113.0	5027.5 ± 111.0	5385.9 ± 65.0
Trisaccharides	116.7 ± 3.9	103.1 ± 0.0	452.4 ± 2.0	621.0 ± 22.2	518.6 ± 39.7	578.6 ± 23.7	2607.0 ± 110.1	2655.9 ± 69.8
Sugar alcohol	520.5 ± 16.0	221.0 ± 18.3	993.7 ± 16.7	961.4 ± 31.0	1578.3 ± 31.0	1442.3 ± 41	2369.4 ± 41.1	3224.4 ± 30.0

A-ambient CO2 ; B- elevated CO2



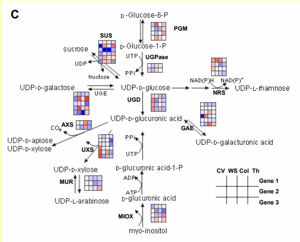


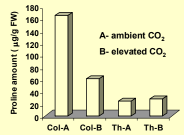
Fig 4 Responses of Sucrose (A), starch (B), and cell wall (C) metabolism to elevated CO_2.

Genes are identified by *Arabidopsis* ID and annotation. Values of change are expressed by color intensity. Up-regulation indicated by shades of blue, down-regulation by red. The scale identifies log_2 at -1 to +1

Asn is detected in Th only in elevated CO_2

Pro is detected in Th and increases strongly in Col-0.

Col-0 alone showed significant up-regulation of P5CDH & P5CS, the enzymes in proline biosynthesis.



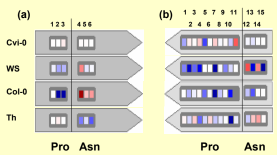


Fig.5 Amino acid metabolism

(a) 1 P5CR, 2 P5CDH, 3 P5CS, 4 Asn2, 5 Asn3, 6 Asn1

(b) 1oxidoreductase, 2 pro oxidase, putative, 3 –6 oxidoreductase, 7 proline oxidase, 8 At3g28480 oxidoreductase, 9 At3g06300 oxidoreductase, 10 At2g17720 oxidoreductase, 11 At2g43080 oxidoreductase, 12 L-asparaginase, putative 13 At5g08100 L-asparaginase, 14 glycosylasparaginas3, 15 - At3g16150 L-asparaginase, putative.

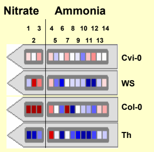


Fig.6 Nitrate and Ammonia metabolism

1 NR1, 2 NR2, 3 NIR1

4 GLU1, 5 GLU2, 6, GSR1, 7 GS2, 8 GS putative, 9 GS putative, 10 GSR2, 11 GS1, 12 GS putative, 13 haloacid dehalogenase-like, 14haloacid dehalogenase-like

Up-regulation indicated by shades of blue, down-regulation by red.

Summary

- With the purpose to study, understand, and model how the plant model *Arabidopsis*, represented by selected ecotypes, behaves in elevated [CO_2] in the field, three *Arabidopsis* and a close relative, Thellungiella halophila, were grown in FACE-rings. Three years (2002, 2003 and 2005) of experiments showed that Arabidopsis can grow well in the field in June, with Fv/Fm recovering within 3 days (2005) after transplanting to the field. Significant differences between treatments and cultivars were consistently observed.
- CO_2 exposure initiated different strategies among the lines with respect to genes related to carbohydrate synthesis and partitioning, cell wall biosynthesis, N-allocation/amino acid metabolism, and stress responses. The preponderance of metabolic functions among the responders in each line seems to indicate that the plants reached an adapted state. Among genes associated with metabolic functions, enzymes in nitrogen/ammonia metabolism, amino acid metabolism, organic acid, and lipid metabolic functions were of note.
- Irrespective of the underlying genetic diversity that distinguished ecotypes, a core set of signature processes and genes reporting atmospheric CO_2 response pathways united *Thellungiella* and the three *Arabidopsis* ecotypes. Genes associated with photosynthesis and chloroplast localization were most highly represented in down-regulated group, while C, N metabolism and redox responses were over-represented among the up-regulated genes.

Reference:

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