# Responses of respiratory rates of *Arabidopsis thaliana* plants grown in elevated $CO_2$

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## Introduction

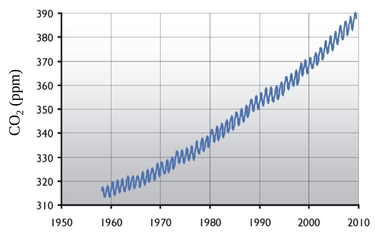



Fig. 1 (left) Atmospheric $CO_2$ concentration measured at Mauna Loa in Hawaii.

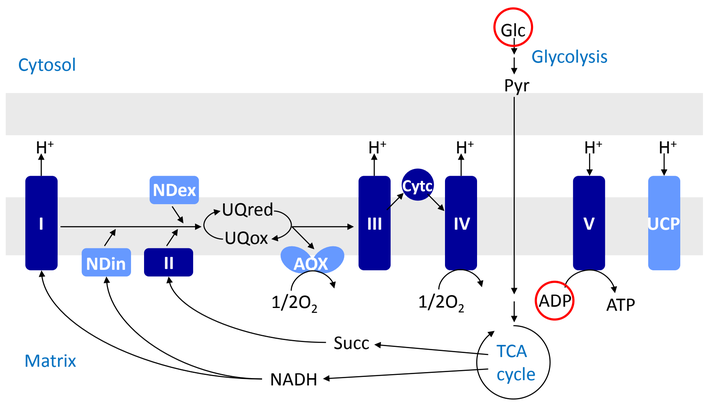



Fig. 2 (right) Respiratory systems in plant. Respiratory rate is limited by amounts of substrates and /or ATP consumption rates.

- Plants can fix carbon via photosynthesis, but they release about half of the fixed carbon via respiration.
- Total carbon release by respiration of terrestrial plants is six-fold amount of artificial release (Amthor 1995, Canadell et al. 2007).
- Photosynthesis rates per leaf area in many species increased in elevated $CO_2$ (Long et al. 2004). On the other hand, responses of respiratory rates of plants grown in elevated $CO_2$ are poorly understood.
- In plants, respiratory rate is generally limited by amounts of substrates and/or ATP consumption rates.

## Materials

*Arabidopsis thaliana* (L.) Heynh. accession Columbia

Growth conditions
- 23℃, 60% of humidity
- Light- 10 h (10:00-20:00)
  100- 130 μmol $m^{-2}$ $s^{-1}$
- $CO_2$ 390 or 780 ppmv

Day 0 ~ 6 → Day 7 → Sampling

Day 0 ~ 6: 30 mL of modified MGRL medium (pH 6, 10 mM $KNO_3$, no sucrose)

Day 7: 40 mL of modified MGRL medium

Sampling: 10 or 2 seedlings $plate^{-1}$


Fig. 3 Plants grown at 390 or 780 ppmv $CO_2$. In elevated $CO_2$, plants grew faster and leaf numbers were more than those in ambient $CO_2$.

- Plants were separated into shoots and roots
- Diurnal changes at day 20
  End of the night: 9:00-10:00
  Noon: 14:00-15:00
  End of the day: 19:00-20:00

**The aims of this study are to examine diurnal changes in respiratory rates, and then to clarify its limiting factor(s) in plants grown in elevated $CO_2$.**

## Results

### 1. Respiratory rates and limiting factors in shoots

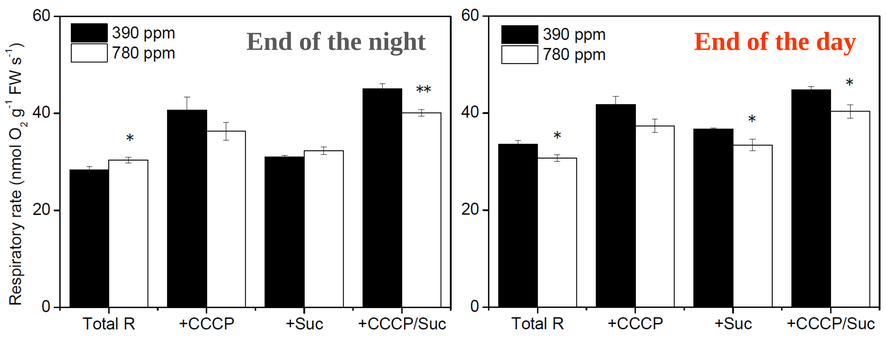
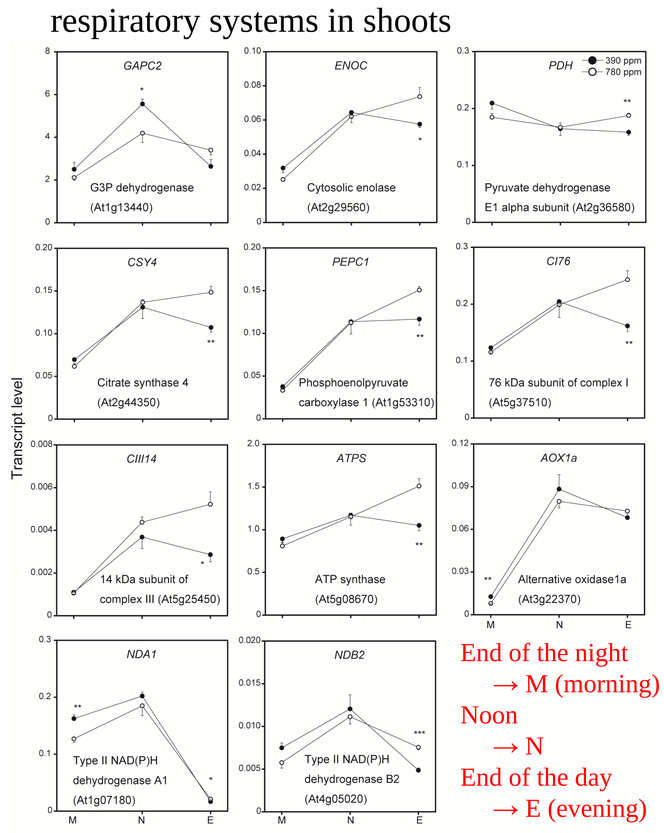



Fig. 4 Respiratory rates before and after addition of sucrose (Suc) and/or carbonyl cyanide m-chloro phenylhydrazone (CCCP). Suc and CCCP were added at a concentration of 100 mM and 10 μM, respectively.

- Total respiratory rates (Total R) at the end of night were higher in shoots in 780 ppmv than in 390 ppmv. At the end of the day, Total R in shoots in 780 ppmv were lower than those in 390 ppmv.
- The capacity of respiration (+CCCP/Suc) was lower in shoots in 780 ppmv than that in 390 ppmv.
- At the end of the night, the respiration rate in 390 ppmv was more enhanced by the additional uncoupler (+CCCP), compared to 780 ppmv.
- There were no significant differences in respiratory rates of roots between the growth $CO_2$ conditions (data not shown).
- These results indicate that ATP consumption rates may differ between the growth $CO_2$ conditions and thereby the respiratory rates showed different responses.

### 2. Transcript levels of genes related to respiratory systems in shoots




End of the night → M (morning)
Noon → N
End of the day → E (evening)

Fig. 5 Diurnal changes of transcript levels of the nuclear-coded gene related to the glycolysis, TCA cycle, and ETC in shoots were analyzed using a Real-time PCR. *RPS15aA* genes were used as an internal standard.

- Expressions of most genes showed diurnal changes in plants grown at both different $CO_2$ conditions.
- Transcription of *ENOC*, *CSY4*, *PEPC1*, *CI76*, *CIII14*, *ATPS*, and *NDB2* showed similar pattern. Their transcript levels increased at noon and then decreased at end of the day. These levels at 780 ppmv $CO_2$ at end of the day were higher than those at 390 ppmv.

### 3. Primary metabolites in shoots

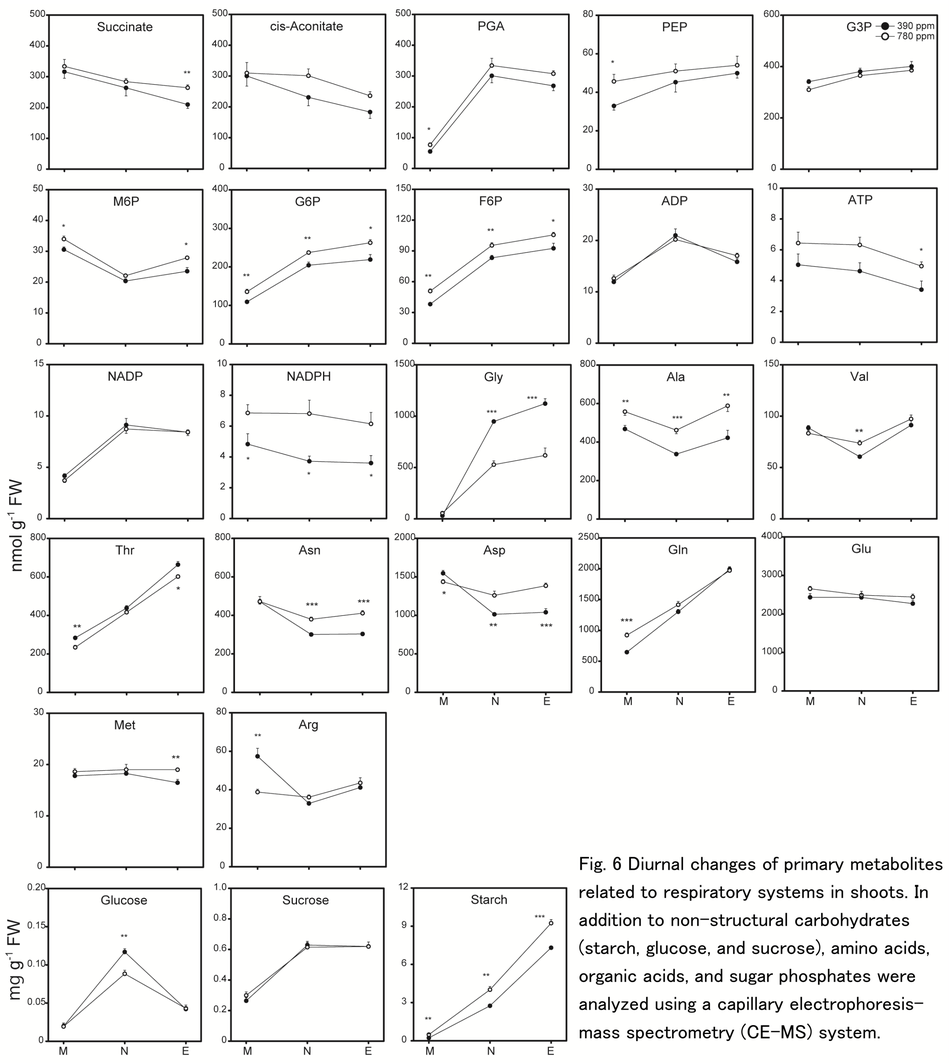



Fig. 6 Diurnal changes of primary metabolites related to respiratory systems in shoots. In addition to non-structural carbohydrates (starch, glucose, and sucrose), amino acids, organic acids, and sugar phosphates were analyzed using a capillary electrophoresis-mass spectrometry (CE-MS) system.

### 4. Responses of primary metabolites and gene expressions related to respiratory systems in elevated $CO_2$

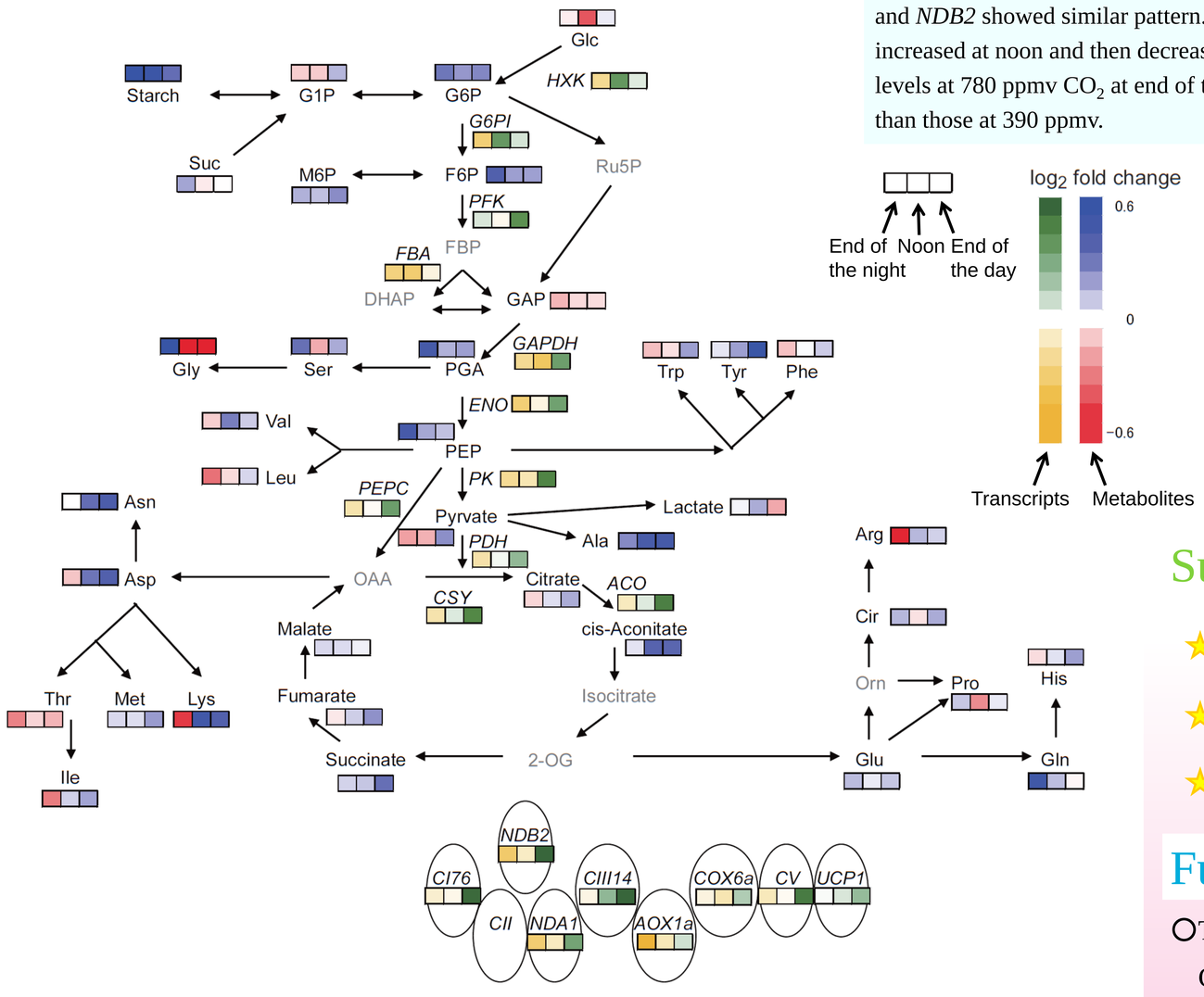






Fig. 7 Heat map of transcripts and primary metabolites. $Log_2$ expression changes in 780 versus 390 ppmv were used. Green or yellow blocks indicate increase or decrease of transcripts. Blue or red indicate increase or decrease of primary metabolites. Gray letters show that the metabolites could not be detected or did not measured.

- Most metabolites showed substantial diurnal changes in both plants.
- The levels of Ala, Val, Asn, Asp, Gln, Glu, Met, Arg, succinate, PEP, *cis*-aconitate, PGA, ATP, M6P, G6P, F6P, and NADPH, were significantly higher in shoots grown at 780 ppmv $CO_2$ than those at 390 ppmv (two-way ANOVA). The levels of Gly, Thr, and G3P were lower in shoots at 780 ppmv $CO_2$ than at 390 ppmv.
- The Gly levels during the day were significantly lower in shoots grown at 780 ppmv than those at 390 ppmv. This denotes that photorespiration was restrained in plants grown at 780 ppmv $CO_2$.
- G6P and F6P levels were higher in shoots at 780 ppmv $CO_2$ than at 390 ppmv.
- The levels of G6P, F6P, and starch were constantly higher throughout the whole day at 780 ppmv $CO_2$ than at 390 ppmv.
- There was no significantly difference in sucrose level between the growth $CO_2$ conditions.

## Summary

- In elevated $CO_2$, the increase in diurnal photosynthetic production influences transcript levels and primary metabolites related to the respiratory system.
- However, there is no direct relationship between these changes and the respiratory rates.
- These results suggest that control of enzyme activities in respiratory systems influences respiratory rates in elevated $CO_2$.

## Future works

- To clarify the relationship between the control of enzyme activities and respiratory rates in elevated $CO_2$, we will measure activity of each enzyme activities in the glycolysis and TCA cycle.
- To investigate whether changes in respiratory systems relate with plant growth, we use some ecotypes of *A. thaliana* showed different responses to elevated $CO_2$.