The role of carbonic anhydrase in C_4 photosynthesis and mesophyll conductance

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Introduction

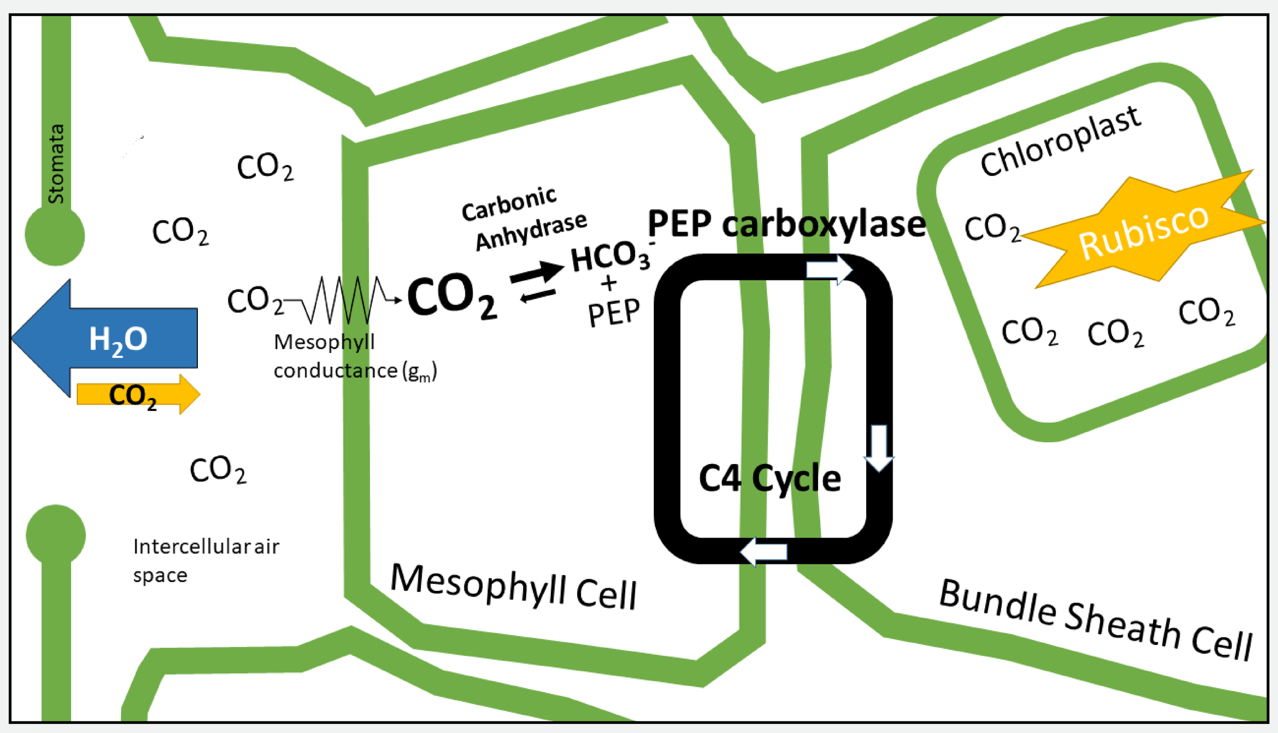

Figure 1: Role of carbonic anhydrase in C_4 photosynthesis.

- C_4 photosynthesis concentrates CO_2 around Rubisco by biochemically shuttling four-carbon acids to bundle sheath cells. Atmospheric CO_2 enters the leaf airspace and diffuses into the mesophyll cells where it is substrate for carbonic anhydrase (CA)
- CA rapidly catalyzes the formation of HCO_3^- which is subsequently fixed by PEP carboxylase (PEPC)
- At ambient [CO_2] and 25°C a maize double knockdown mutant of the two mesophyll CA genes (*ca1ca2*) did not limit photosynthesis despite *ca1ca2* plants having 3% of wild-type CA activity (Studer et al.,2014).
- The role of CA in the conductivity of CO_2 movement into mesophyll cells (mesophyll conductance– g_m) in C_4 plants is unknown.
- Methods to measure g_m in C_4 plants are influenced by CA. Therefore, the *ca1ca2* mutants provide the unique opportunity to evaluate the sensitivity of g_m methods.

Objectives

1. Determine if CA limits photosynthesis at elevated temperatures during C_4 photosynthesis
2. Quantify the contribution of CA to substrate availability for C_4 photosynthesis
3. Determine the role of CA in C_4 mesophyll CO_2 conductance

Materials and Methods

- Wild-type and *ca1ca2* plants were grown under elevated CO_2 (1%), 500 µmol m^{-2} s^{-1} PPFD, 16 hr days, and 31/28°C day/night regime
- Assimilation vs intercellular CO_2 (A/Ci) curves were measured on *ca1ca2* and wild-type maize plants from 10 to 40°C at 5°C increments
- Biochemical assays of Rubisco, PEPC, and CA activity were measured *in vitro* on crude leaf extract
- A/C_i curves at 15, 25, and 35°C were coupled with on-line measurements of $\Delta^{13}CO_2$, $\Delta^{18}CO_2$ and $\delta^{18}O$ of transpired water

Results

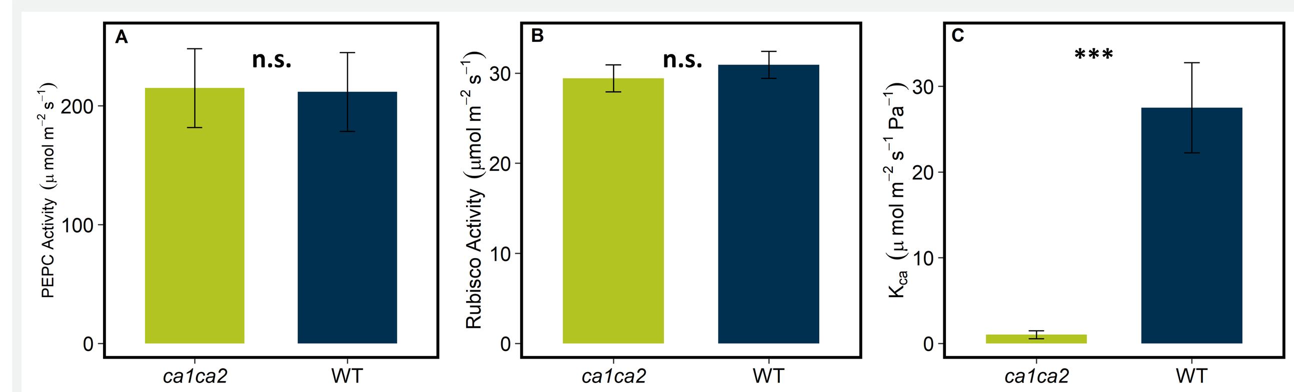

Figure 2: Mean *in vitro* enzyme activity for *ca1ca2* and WT plants (n=8) for A) PEPC, B) Rubisco, and C) the rate constant for carbonic anhydrase at 25°C. *** statistically significant difference from t-tests at alpha = 0.05.

- *ca1ca2* disruptions only reduced CA activity

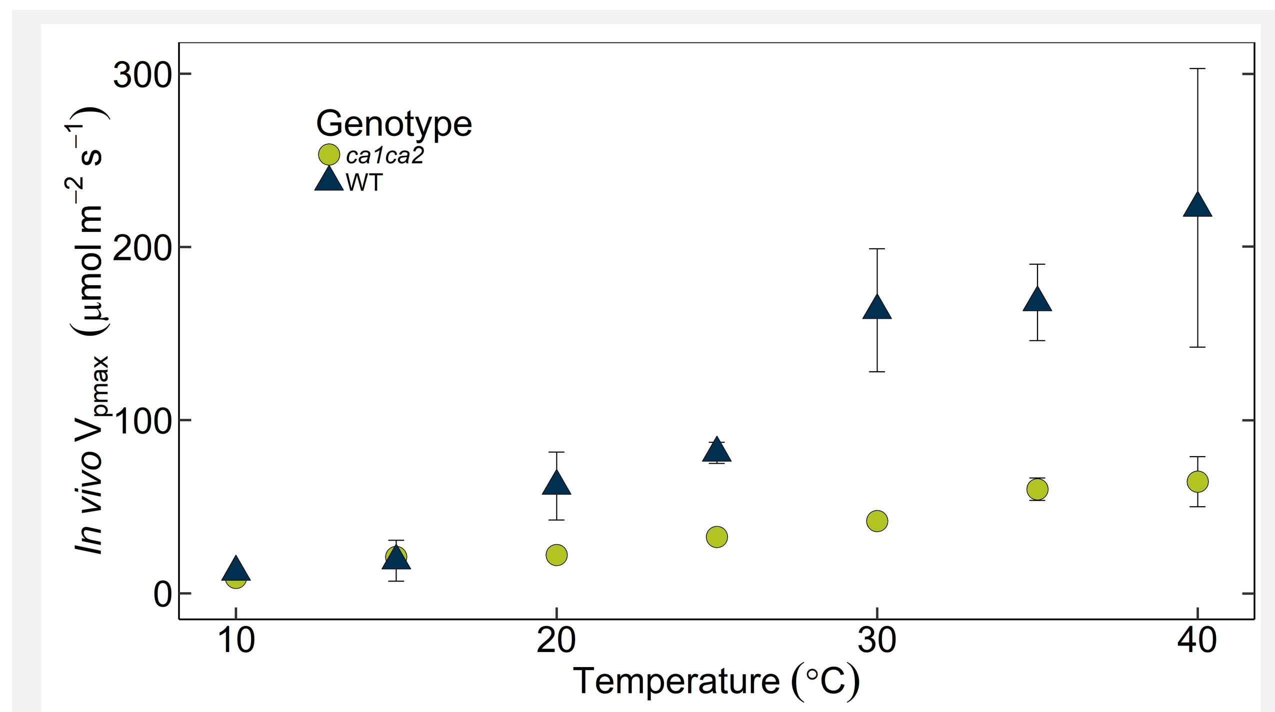

Figure 3: *ca1ca2* and WT *in vivo* Vp_{max} calculated from 10 to 40°C by solving for the initial slope of A/Ci curves modeled with the temperature responses of C_4 photosynthetic parameters from Boyd et al., 2015. Infinite g_m assumed. n= 4

- Suggests PEPC limitation at higher temperatures in *ca1ca2* plants
- Differences could be attributed to CO_2, HCO_3^-, or PEPC

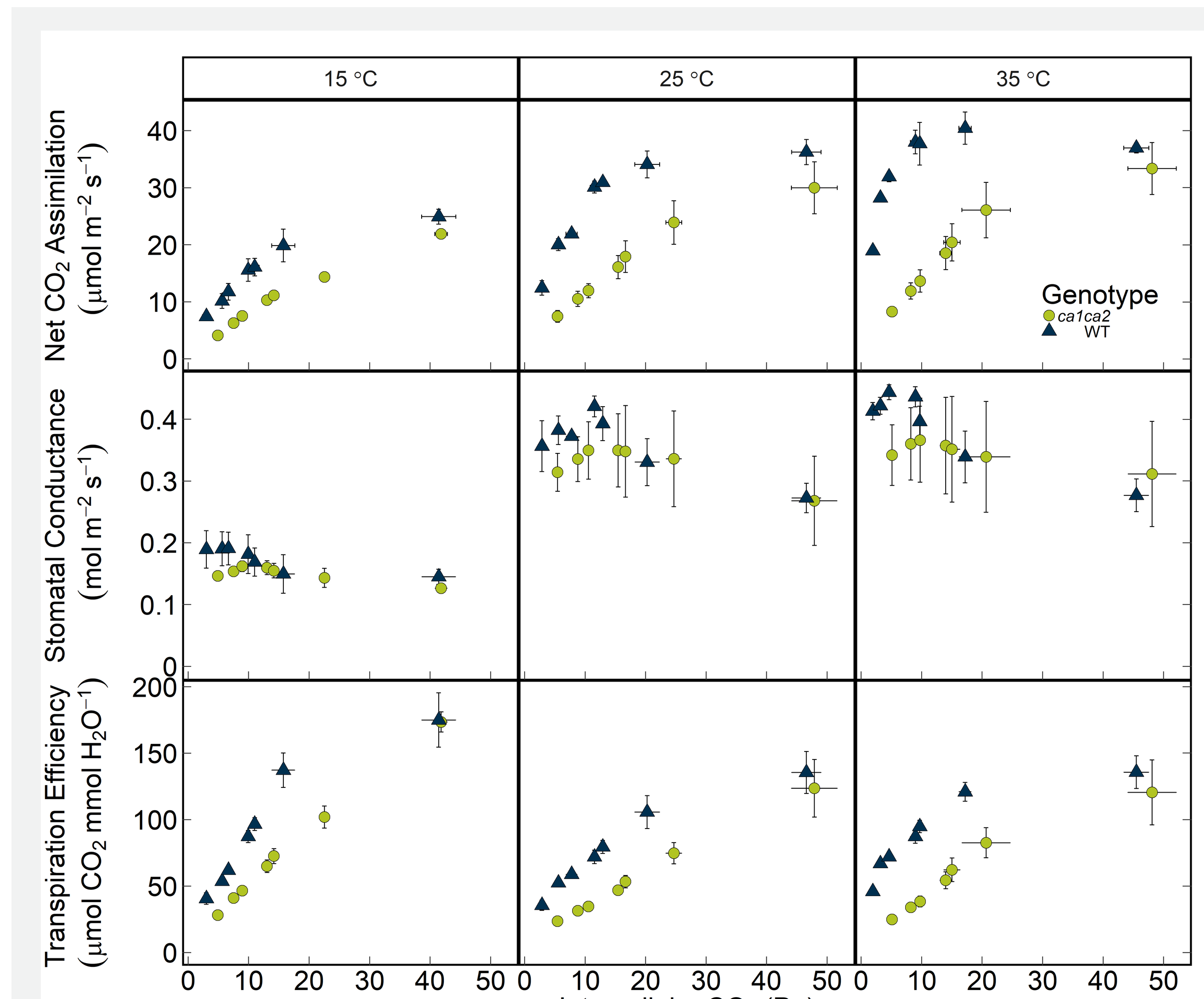

Figure 4: Gas exchange measurements vs Ci curves for *ca1ca2* and WT at 15, 25, and 35°C. A) Net CO_2 assimilation rate, B) stomatal conductance, and C) transpiration efficiency calculated as net CO_2 assimilation/stomatal conductance.

- CA mutants lose more water per CO_2 fixed because of reduced assimilation rate, not stomatal conductance

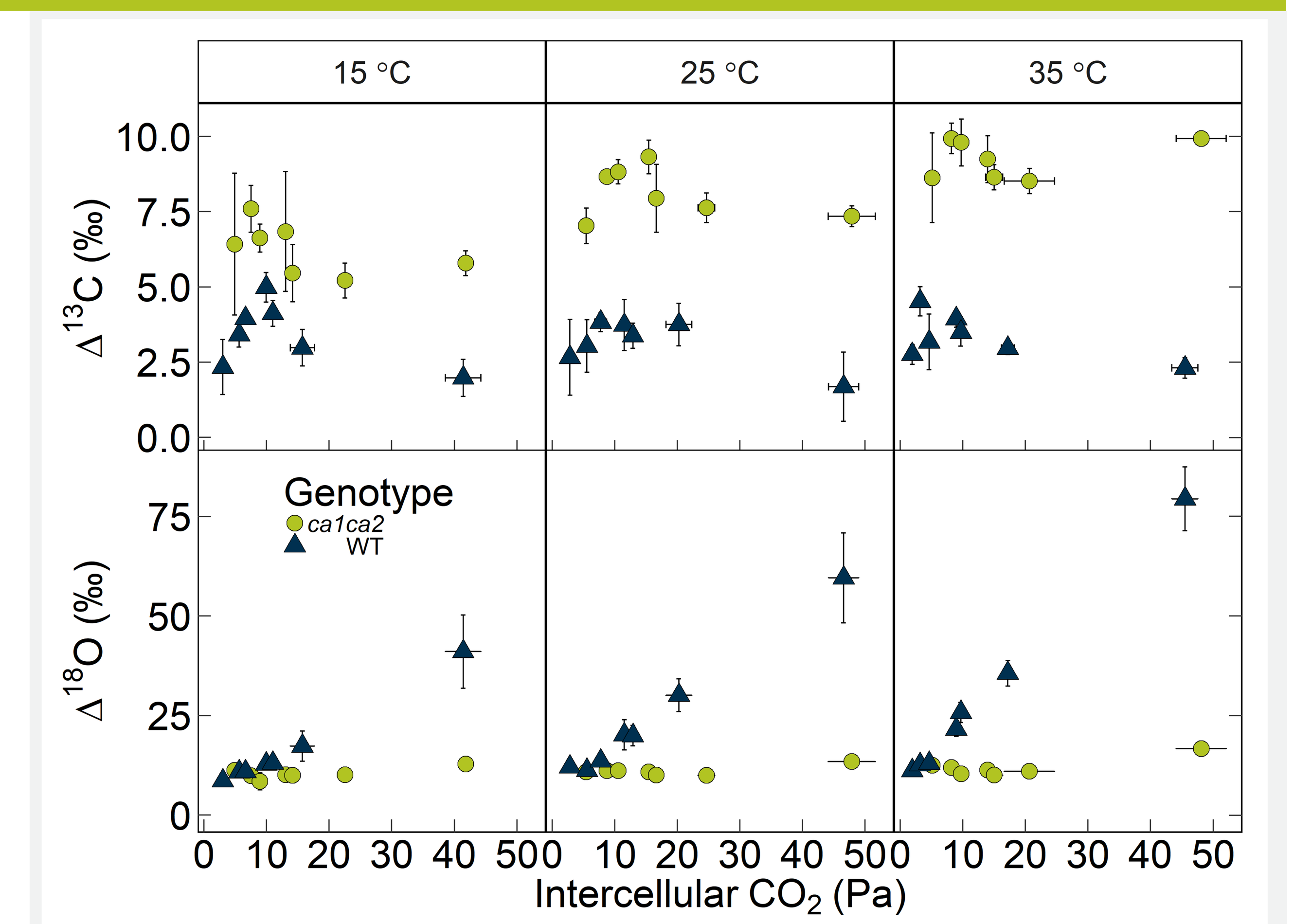

Figure 5: Gas exchange and online measurements of leaf $\Delta^{13}CO_2$ and $\Delta C^{18}O_2$.

- Differences in $\Delta^{13}C$ suggest that lack of CA increases rates of PEPC activity to CO_2 hydration by CA and limits HCO_3^- supply to PEPC
- Differences in $\Delta^{18}O$ suggest that CO_2 and cytosolic H_2O are not in isotopic equilibrium

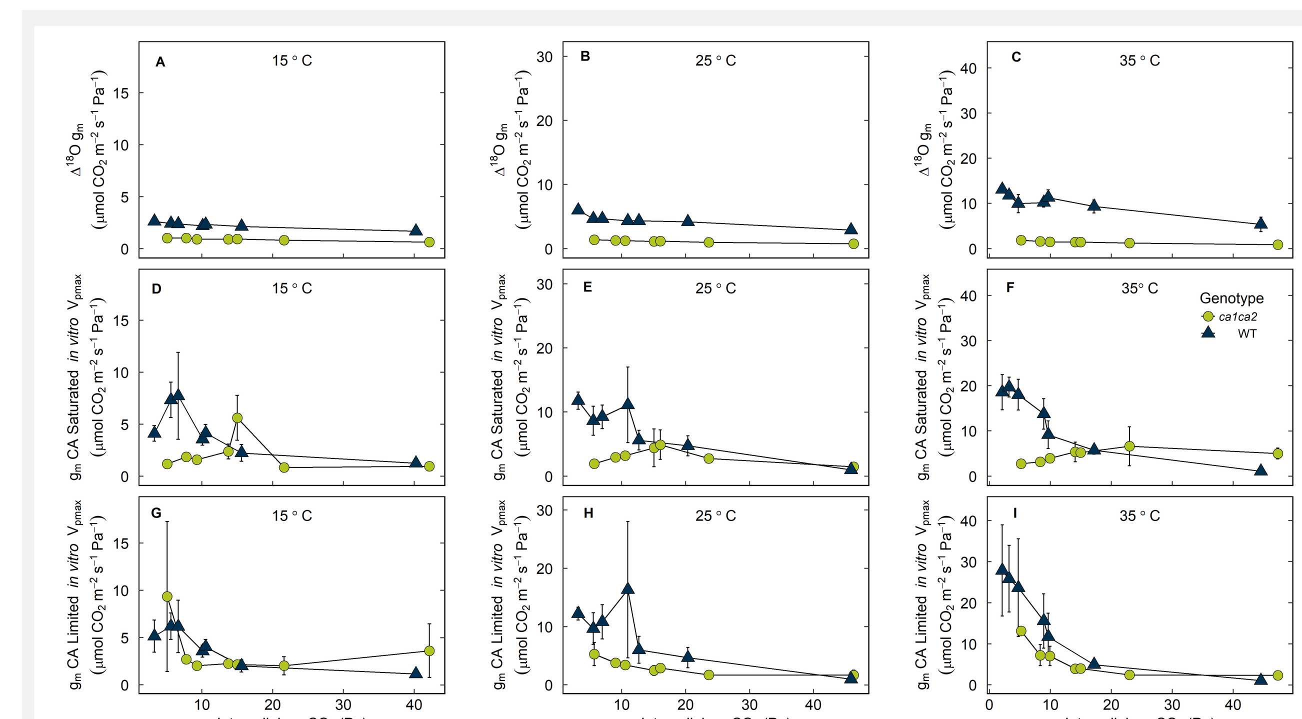

Figure 6: Mesophyll conductance measurements with increasing temperature and pCO_2 on *ca1ca2* and WT plants. $\Delta^{18}O$-g_m (A-C), *in vitro* V_{Pmax} CA Saturated-g_m (D-F), and *in vitro* V_{Pmax} CA Limited-g_m (G-I)

- $\Delta^{18}O$-g_m method is sensitive to CA activity and drastically underestimates g_m
- CA exhibits little effect on calculated g_m when modeled with *in vitro* enzyme data

Conclusions

- CA limits C_4 photosynthesis more at higher temperatures by substrate limitation to PEPC
- CA is not a large component of mesophyll conductance in C_4 maize
- Methods to measure g_m in C_4 plants should cautiously consider the effects of CA on HCO_3^- availability