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Acclimation of photosynthesis to temperature in *Arabidopsis thaliana* and *Brassica oleracea*

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Abstract

Plants differ in how much the response of net photosynthetic rate (P_N) to temperature (T) changes with the T during leaf development, and also in the biochemical basis of such changes in response. The amount of photosynthetic acclimation to T and the components of the photosynthetic system involved were compared in *Arabidopsis thaliana* and *Brassica oleracea* to determine how well *A. thaliana* might serve as a model organism to study the process of photosynthetic acclimation to T. Responses of single-leaf gas exchange and chlorophyll fluorescence to CO_2 concentration measured over the range of 10−35 °C for both species grown at 15, 21, and 27 °C were used to determine the T dependencies of maximum rates of carboxylation (V_{Cmax}), photosynthetic electron transport (J_{max}), triose phosphate utilization rate (TPU), and mesophyll conductance to carbon dioxide (g'_m). In *A. thaliana*, the optimum T of P_N at air concentrations of CO_2 was unaffected by this range of growth T, and the T dependencies of V_{Cmax}, J_{max}, and g'_m were also unaffected by growth T. There was no evidence of TPU limitation of P_N in this species over the range of measurement conditions. In contrast, the optimum T of P_N increased with growth T in *B. oleracea*, and the T dependencies of V_{Cmax}, J_{max}, and g'_m, as well as the T at which TPU limited P_N all varied significantly with growth T. Thus *B. oleracea* had much a larger capacity to acclimate photosynthetically to moderate T than did *A. thaliana*.

Additional key words: chlorophyll fluorescence; mesophyll conductance to CO_2; photosynthetic electron transport; species differences; triose phosphate utilization ratio.

Introduction

With the increasing use of biochemically based models of C_3 photosynthesis in diverse applications, there has been renewed interest in the temperature (T) acclimation of net photosynthetic rate (P_N) and in determining which model parameters vary with growth T and in what species they vary (*e.g.* Bunce 2000, Medlyn *et al.* 2002a,b, Hikosaka *et al.* 2006, Kattge and Knorr 2007). Knowledge of the mechanisms by which photosynthetic systems acclimate to T would add a predictive capacity not currently available. *Arabidopsis thaliana* has proven an extremely useful model species for elucidating such mechanisms, but its suitability for studies of T acclimation of P_N is unclear. *A. thaliana* showed increased P_N during the development of freezing tolerance, which was related to rates of sucrose synthesis (Strand *et al.* 2003), and Kim and Portis (2005) examined the decrease in P_N caused by high T in *A. thaliana* lines with modified membrane composition, but the acclimation of P_N to T has not been fully documented in this species. In this study I compared characteristics of the T acclimation of P_N in *A. thaliana* with *Brassica oleracea*, another member of the Brassicaceae also adapted to cool conditions.

The widely used biochemically based model of C_3 photosynthesis of Farquhar *et al.* (1980) uses a maximum rate of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) carboxylation (V_{Cmax}), and a maximum rate of photosynthetic electron transport (J_{max}), along with kinetic characteristics of RuBPCO, respiration, and the CO_2 compensation concentration in the absence of respiration to predict P_N as a function of substomatal CO_2 concentration (C_i). It was recognized that V_{Cmax} and J_{max} likely varied with species and with growth conditions,

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Abbreviations: C_i – $[CO_2]$ in the intercellular airspace; C_c – $[CO_2]$ at ribulose-1,5-bisphosphate carboxylase/oxygenase; Chl – chlorophyll; g'_m – mesophyll conductance to CO_2; J – rate of photosynthetic electron transport; J_{max} – maximum rate of photosynthetic electron transport; K_C – Michaelis constant for CO_2; K_O – Michaelis constant for O_2; LED – light emitting diode; P_N – net photosynthetic rate; PPFD – photosynthetic photon flux density; RuBPCO – ribulose-1,5-bisphosphate carboxylase/oxygenase; T – temperature; TPU – triose phosphate utilization; V_{Cmax} – the maximum rate of carboxylation of RuBPCO; ΔH_a – energy of activation.

and that the ratio of J_{max} to V_{Cmax} would affect the *T* dependence of P_N (Farquhar and Caemmerer 1982). While some surveys have found that the ratio of J_{max} to V_{Cmax} decreases with increasing growth temperature (Bunce 2000, Kattge and Knorr 2007), changes in the temperature dependencies of V_{Cmax} and J_{max} with growth *T* have often been found to have a more important role in *T* acclimation of P_N than changes in their ratio (Bunce 2000, Ziska 2001, Hikosaka *et al.* 2006, Kattge and Knorr 2007).

Since the initial development of the Farquhar C_3 photosynthesis model, the importance to the modeling of C_3 photosynthesis of two other phenomena has become recognized. One is the limitation imposed on P_N by the rate of triose phosphate utilization (TPU) (Sharkey 1985). While seldom of importance except at very cool *T* at current atmospheric levels of CO_2, TPU limitation may become more common as atmospheric CO_2 increases (Sage and Kubien 2007). TPU limitation has been added to C_3 photosynthesis models (Harley and Sharkey 1991), and Sage and Kubien (2007) reviewed evidence that an increase in TPU at low growth *T* can be an important part of acclimation of P_N to low *T*. In *A. thaliana*, cold hardening involves increased rates of sucrose synthesis (Strand *et al.* 2003), possibly removing a TPU limitation, although TPU limitation was not assayed in that study.

The other phenomenon of recently recognized importance to the modeling of P_N is the decrease in [CO_2] between the substomatal air space and the site of carboxylation inside the chloroplast. Early versions of the C_3 photosynthesis model assumed that there was a negligible gradient of [CO_2] from inside the stomata to the site of carboxylation (Farquhar *et al.* 1980). This was later shown to be incorrect (*e.g.* Evans *et al.* 1986), but the "mesophyll resistance" was initially thought to be a physical diffusive resistance. The involvement of carbonic anhydrase (Flexas *et al.* 2006) and aquaporins (Terashima and Ono 2002) in mesophyll conductance (g'_m), as well as its strong *T* dependency (Bernacchi *et al.* 2002, Warren and Dryer 2006, Yamori *et al.* 2006a) argue against a purely physical diffusive resistance. Mesophyll conductance has been estimated by carbon isotope discrimination, and by combined analysis of chlorophyll (Chl) fluorescence signals and leaf gas exchange (*e.g.* Loreto *et al.* 1992). Yamori *et al.* (2006a) found differences in the *T* response of g'_m in spinach grown at two *T*s, which contributed to the differences in the optimum *T* of P_N. The magnitude of g'_m has been estimated in *A. thaliana* in relation to leaf age (Flexas *et al.* 2007b), but possible involvement of g'_m in *T* acclimation has not been investigated in this species.

In this study, responses of single leaf gas exchange and Chl fluorescence to CO_2 concentration were measured over the range of 10 to 35 °C for *A. thaliana* and *B. oleracea* grown over a range of moderate *T*s. These measurements were used to determine the *T* dependencies of V_{Cmax}, J_{max}, TPU, and g'_m in order to characterize and compare the acclimation of P_N to *T* in these species.

Materials and methods

Arabidopsis thaliana (L.) Heynh. ecotype Columbia, and *Brassica oleracea* var. *acephala* DC. (Collards) cv. Georgia Southern were grown separately in controlled environment chambers kept at constant temperatures of 15, 21, or 27 °C. Plants were grown one to a pot in plastic pots filled with vermiculite. Pots were flushed every day (*B. oleracea*) or every other day (*A. thaliana*) with a complete nutrient solution containing 3.6 mM nitrogen. The chambers provided 14 h per day of irradiation from a mixture of high pressure sodium and metal halide lamps at 900 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD). The carbon dioxide concentration [CO_2] was maintained between 370 and 390 μmol mol^{-1} by addition of pure CO_2 or air scrubbed of CO_2 under the control of an absolute infrared CO_2 analyzer.

Gas exchange and Chl fluorescence measurements were conducted on single, intact mature leaves. In *A. thaliana*, these were the largest leaves on plants which had just begun elongation of the flowering stalk. In *B. oleracea* measurements were made on third leaves which had finished growth in area a few days prior. Tests showed that these leaves had very similar P_N a few days before and after these stages of development. Gas exchange and fluorescence measurements were conducted using a *CIRAS-2* portable photosynthesis system (*PP Systems*, Amesbury, MA, USA), and *OS-500* PAM fluorometer (*Opti-Sciences*, Tyingsborro, MA, USA), respectively. The photosynthesis system was completely inside a controlled environment chamber, whose *T* was controlled to be the same as that of the measured leaf. This prevented problems with condensation within the photosynthesis system, and control of humidity in the chamber allowed leaf to air water vapor pressure differences to be kept between 1.0 and 1.5 kPa at all leaf *T*s. Two fluorescence probes were inserted into a custom-adapted leaf cuvette without shading the leaf, and comparisons were made of the steady state and maximal photosystem 2 fluorescence values obtained from the upper surface (irradiated by "white" LEDs at 1 500 μmol m^{-2} s^{-1} PPFD) and from the lower surface.

Gas exchange and fluorescence measurements were begun at the lowest temperature (10 °C). Leaves were first equilibrated at an incoming [CO_2] of about 400 μmol mol^{-1} at high PPFD (1 500 μmol m^{-2} s^{-1}) until P_N was stable. The [CO_2] external to the leaf was 375±5 μmol mol^{-1} with this incoming [CO_2]. Respiration and the CO_2 compensation concentration in the absence of respiration were then determined by the Laisk method (Flexas *et al.*

2007a), which uses the intersection of linear (low C_i) portions of P_N *vs*. C_i curves at a range of PPFDs. After returning leaves to the initial conditions, P_N *versus* C_i curves at high PPFD were determined using six concentrations of incoming [CO$_2$], approximately 100, 200, 400, 600, 800, and 1 000 μmol mol^{-1}, followed by a third measurement at 400 μmol mol^{-1} to determine reversibility. The leaf and chamber *T*s were then increased by 5 °C and the sequence of measurements was repeated up to 35 °C.

At each measurement *T*, fluorescence measurements were used to determine the quantum yield of electron transport at each [CO$_2$]. Fluorescence and P_N measurements at 2 % O$_2$ at 25 °C were used to determine the proportionality between electron transport and fluorescence (Harley *et al*. 1992). The g'_m to CO$_2$ was estimated using both the variable and constant J methods (Harley *et al.* 1992). Measurements at 100−400 μmol mol^{-1} [CO$_2$] were used for the variable J method, and rates at 600–1 000 μmol mol^{-1} [CO$_2$] were used for the constant J method because the fluorescence signal did not change with [CO$_2$] over that range of values. For the variable J method, two estimates of g'_m were obtained at each [CO$_2$], one based on the fluorescence signal from the upper leaf surface and one based on the signal from the lower leaf surface. The constant J method sometimes did not yield a result (Harley *et al.* 1992), especially when P_N decreased at the highest C_i values, which is indicative of TPU limitation of P_N (Sharkey 1985). For each species and growth *T*, gas exchange and fluorescence measurements were conducted on 3 or 4 leaves, each from a different plant.

Using the kinetic characteristics of the Michaelis constants (K_C and K_O) for CO$_2$ and O$_2$ of RuBPCO, respectively, given in Sharkey *et al.* (2007), and the measured values of respiration in the light and the CO$_2$ compensation concentration in the absence of respiration, V_{Cmax} based on C_i at each *T* was estimated from P_N and C_i at the two lowest values of C_i, and J_{max} was estimated from P_N at high C_i values, but excluding those with TPU limitation. At each value of C_i, the [CO$_2$] at the site of fixation by RuBPCO (C_c), was calculated from P_N and g'_m estimated by the constant J method (see later), and V_{Cmax} based on C_c was calculated.

The *T* dependency of V_{Cmax} was summarized by its activation energy (ΔH_a) (Bernacchi *et al*. 2001), rather than by an Arrhenius function (Kattge and Knorr 2007), because plots of ln(V_{Cmax}) *versus T* were linear up to the highest *T*. The *T* dependencies of J_{max} were summarized by fitting the equation recommended by June *et al.* (2004), which is a three parameter Gaussian curve:

$$J_{max} = a\, e^{[-0.5\,((x - xo)/b)2]},$$

where a is the value of J_{max} at the optimum *T*, b is the steepness of the curve, and x_o is the optimum *T*. Curve fitting was done using the *Sigma Plot* v. *9* regression utility. Limitation of P_N by TPU was identified by either a lack of increase of P_N with C_i or a decrease in P_N with C_i (Sharkey 1985).

Results

The optimum temperature of P_N at air levels of [CO$_2$] did not vary among the three growth *T*s in *A. thaliana*, but increased with growth *T* in *B. oleracea* from 25 °C for plants grown at 15 °C to at least 35 °C for plants grown at 27 °C (Fig. 1). In *A. thaliana*, growth at the highest *T* reduced rates at all measurement *T*s, but the *T* response curves for the three growth *T*s were nearly parallel. In *B. oleracea*, the shape of the *T* response curves also differed with growth condition, with flatter curves at lower growth *T*s. At low measurement *T*s, highest P_N were achieved by the *B. oleracea* plants grown at the lowest *T*, and at high measurement *T*s, highest rates were achieved by the plants grown at the highest *T* (Fig. 1*A,B*).

Estimates of g'_m using the variable J method did not differ significantly over the external [CO$_2$] range of 100−400 μmol mol^{-1} (not shown), so mean values were used. At the lowest growth *T*, estimates of g'_m using fluorescence from the upper (irradiated) surface and the lower surface differed substantially at the higher measurement *T*s (Fig. 1*C,D*). As also illustrated in Fig. 1, g'_m values estimated with the variable J method were very low at low measurement *T*s compared with estimates using the constant J method. This was true at all growth *T*s in both species. Because g'_m estimated with the variable J method at low *T*s resulted in very low values of C_c and unrealistically high values of V_{Cmax} at low *T*, V_{Cmax} based on C_c was only calculated using g'_m estimated with the constant J method. In *A. thaliana*, g'_m estimated with the constant J method did not vary significantly with either growth or measurement *T* (Table 1). In *B. oleracea*, g'_m increased linearly with measurement *T*, and the slope of the response increased with growth *T* (Table 1). P_N calculated under the assumption that g'_m was infinite still indicated a higher optimum *T* of P_N for *B. oleracea* plants grown at 27 than at 15 °C (not shown).

The activation energy of V_{Cmax} did not vary with growth *T* in *A. thaliana*, whether V_{Cmax} was estimated from C_i or from C_c (Table 1). In *B. oleracea* the activation energy of V_{Cmax} increased significantly with growth *T* whether calculated from C_i or from C_c (Table 1). Using C_i or C_c to estimate V_{Cmax} did not substantially affect the activation energy of V_{Cmax} in either species. V_{Cmax} at 25 °C estimated either from C_i or from C_c was lower in *A. thaliana* plants grown at 27 °C than at the other *T*s, but was only slightly reduced by increasing growth *T* in *B. oleracea* (Fig. 2).

The optimum *T* for J_{max} did not vary with growth *T* in *A. thaliana*, but increased significantly with growth *T*

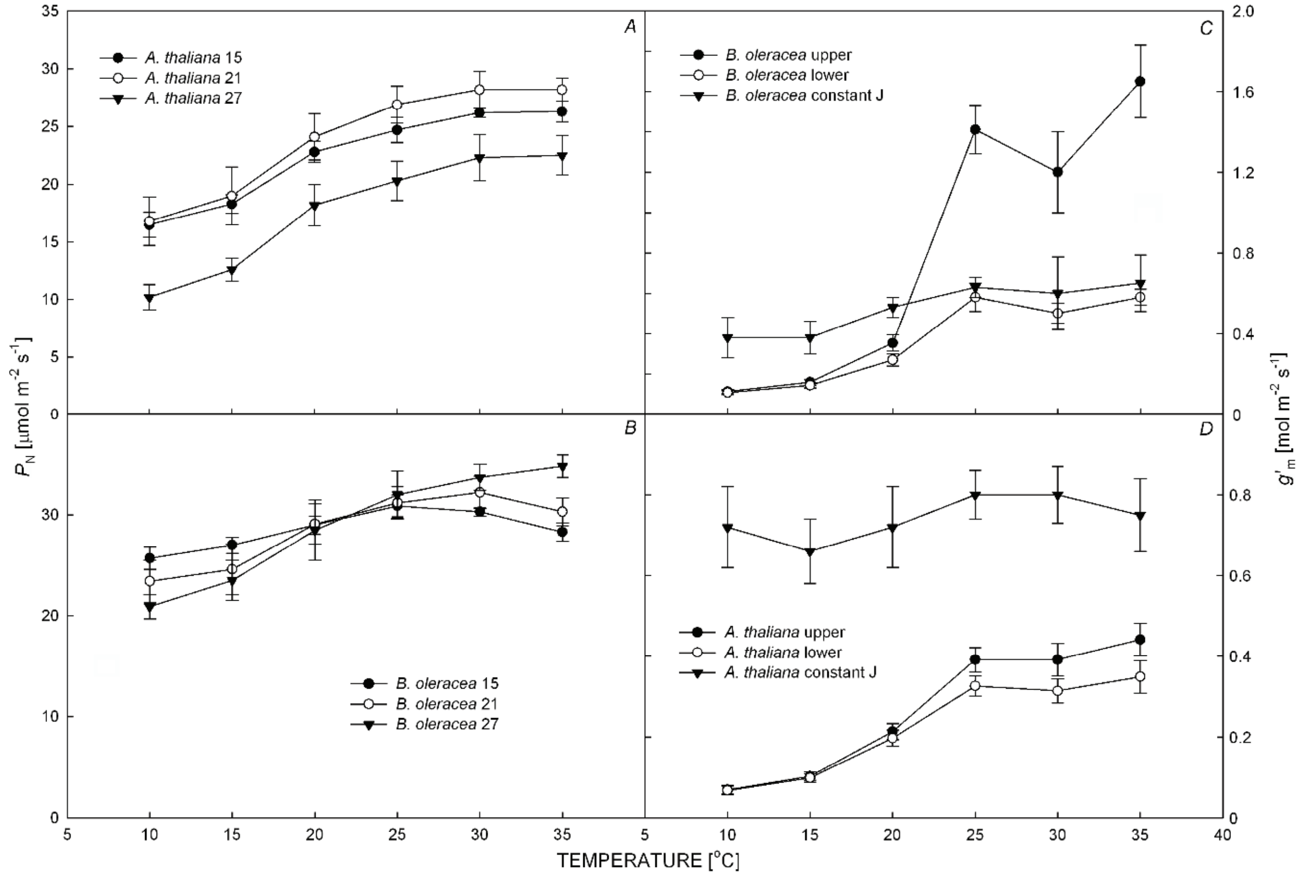

Fig. 1. Responses of (*A, B*) net photosynthetic rate (P_N) and (*C, D*) mesophyll conductance to CO_2 (g'_m) to measurement temperature in *Arabidopsis thaliana* and *Brassica oleracea* grown at (*A, B*) 15, 21, and 27 °C or (*C, D*) 15 °C. (*A, B*): Measurements were made at saturating PPFD, with an external [CO_2] of about 375 µmol mol^{-1}, and a leaf to air water vapor pressure differences of 1.0−1.5 kPa. (*C, D*): Estimates of g'_m were made from gas exchange measurements and fluorescence signals from either the upper or lower leaf surface using the variable or the constant J method. *Vertical bars* represent S.E. for $n = 3$ or 4.

Table 1. Mesophyll conductance to CO_2 (g'_m) estimated using the constant J method over the temperature range of 10 to 35 °C for *A. thaliana* and *B. oleracea* grown at three temperatures. In *A. thaliana*, g'_m did not vary significantly with measurement temperature, so mean values are given. In *B. oleracea*, g'_m varied linearly with temperature and the linear regressions are given. Also shown are activation energies (ΔH_a) characterizing the response of V_{Cmax} to measurement temperature over the range of 10 to 35 °C. V_{Cmax} was estimated from C_i values or from C_c values. Values are ±S.E. for $n = 3$ or 4.

Growth temperature [°C]	g'_m *A. thaliana* mean	 *B. oleracea* intercept	 slope	 r^2	ΔH_a *A. thaliana* C_i	 C_c	 *B. oleracea* C_i	 C_c
15	0.71±0.19	0.28±0.09	0.012±0.004	0.35	64.2±3.0	67.9±2.9	50.9±3.9	51.7±3.8
21	0.81±0.14	0.14±0.20	0.029±0.008	0.34	65.7±2.2	67.2±2.2	56.8±3.7	56.1±3.9
27	0.65±0.14	−0.45±0.28	0.062±0.012	0.64	67.2±2.0	68.6±2.2	64.9±1.5	61.3±2.0

in *B. oleracea* from about 30 °C for plants grown at 15 °C to about 37 °C for plants grown at 27 °C (Table 2). The J_{max} value at the optimum *T* for J_{max} was lower for the highest growth *T* in *A. thaliana*, but increased with growth *T* in *B. oleracea* (Table 2). The parameter quantifying the steepness of the curves was similar for both species and for all growth *T*s (Table 2).

TPU limited P_N of *B. oleracea* at high C_i at the 10 °C measurement *T* when plants were grown at 21 and 27 °C, but not for plants grown at 15 °C (Fig. 3). TPU never limited P_N in *A. thaliana* over the observed range of measurement [CO_2] or *T* conditions for any growth *T*. This is illustrated in Fig. 3 for plants grown at the warmest *T* and measured at the lowest *T*. In *B. oleracea*, TPU limited P_N at high C_i at the measurement *T*s of 10 and 15 °C, but not at higher *T*s, for plants grown at both

21 and 27 °C, but never limited P_N for plants grown at 15 °C.

The ratio of J_{max} to V_{Cmax} at 25 °C did not vary significantly with growth *T* in either species. The mean was 1.36±0.02 for *A. thaliana* and 1.30±0.02 in *B. oleracea* when V_{Cmax} was calculated from C_i, and 1.20±0.06 and 1.09±0.02, respectively, for the two species when V_{Cmax} was calculated from C_c.

Discussion

Both *A. thaliana* and *B. oleracea* had decreasing V_{Cmax} with increasing growth *T*, which is typical of plants adapted to cool climates (Bunce 2000). Neither species acclimated photosynthetically to growth *T* by an altered J_{max}/V_{Cmax}, which can change the optimum *T* of P_N (Farquhar and Caemmerer 1982). Kattge and Knorr (2007) concluded that a change in the ratio measured at a constant *T* was common and an important component of photosynthetic acclimation to *T*. However, Hikosaka *et al.* (2006) stated no consistent response of the J_{max}/V_{Cmax} to growth *T*, nor did Bunce (2000) in comparing eight species. Of the two species studies here, only *B. oleracea* showed any modification of the *T* dependencies of the photosynthetic parameters V_{Cmax}, J_{max}, and g'_m in response to growth *T*.

A shift in the optimum *T* of J_{max} with growth *T* as observed here in *B. oleracea* is common and not controversial (*e.g.* Bunce 2000, Ziska 2001, June *et al.* 2004, reviewed in Medlyn *et al.* 2002a, Hikosaka *et al.* 2006, Kattge and Knorr 2007), although clearly not universal, as it did not occur in *A. thaliana*. The biochemical basis of the shift remains uncertain (Hikosaka *et al.* 2006). My results for *B. oleracea* closely resemble those occurring in soybean (June *et al.* 2004), with a shift in the optimum *T* but no substantial change in the value of the parameter defining the steepness of the curve.

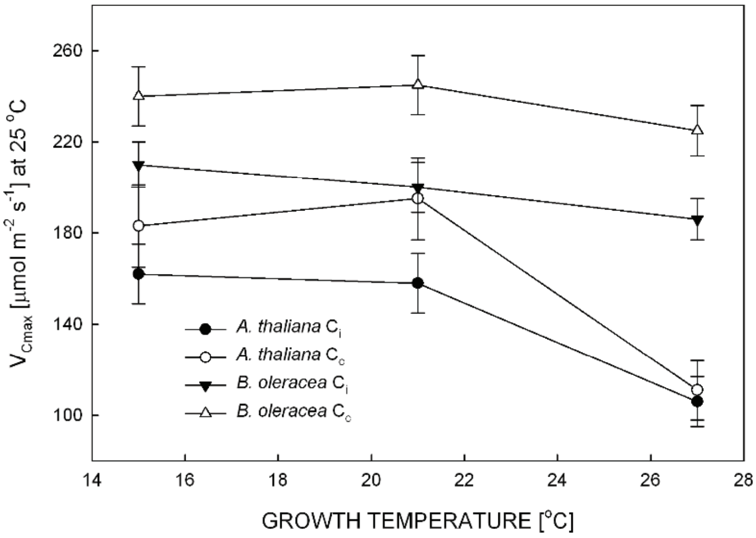

Fig. 2. Estimates of the maximum rate of carboxylation (V_{Cmax}) at 25 °C of *Arabidopsis thaliana* and *Brassica oleracea* grown at three temperatures. V_{Cmax} was estimated assuming the [CO_2] at the site of fixation was the same as that in the intercellular air space (C_i) or was C_c. *Vertical bars* represent S.E. for *n* = 3 or 4.

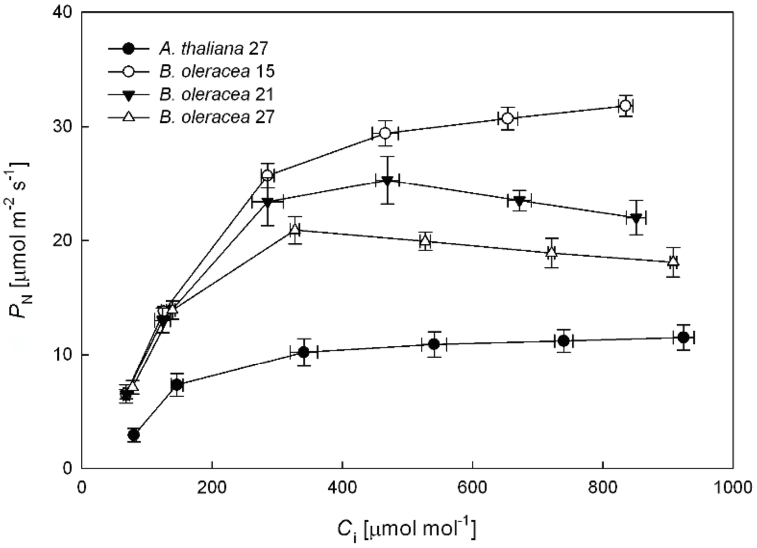

Fig. 3. Net photosynthetic rate (P_N) at different substomatal [CO_2] (C_i) for *Brassica oleracea* grown at 15, 21, and 27 °C and for *Arabidopsis thaliana* grown at 27 °C. All measurements were made at 10 °C and saturating PPFD. *Bars* represent S.E. for *n* = 3 or 4.

Table 2. Responses of J_{max} to growth temperature [ºC] in *A. thaliana* and *B. oleracea* grown at three temperatures. Responses were fit to the Gaussian 3 parameter curve: J_{max} [μmol m^{-2} s^{-1}] = $a\ e^{[-0.5\ ((x - xo)/b)2]}$, where a is the value of J_{max} at the optimum temperature, b is the steepness of the curve, and x_o is the optimum temperature.

Species	Growth temperature	a	b	x_o
A. thaliana	15	283±13	18.1±2.1	38.0±2.9
	21	288±18	18.1±2.0	39.6±3.3
	27	210±17	16.9±2.3	39.5±3.9
B. oleracea	15	282±5	16.4±1.0	30.2±0.9
	21	303±8	16.8±1.6	34.1±2.1
	27	317±10	16.8±1.2	37.3±1.7

Changes in the apparent activation energy of V_{Cmax} with growth *T* such as observed here in *B. oleracea* are somewhat controversial. The review by Hikosaka *et al.* (2006) concluded that increases in activation energy of V_{Cmax} with increased growth *T* were the most consistent aspect of photosynthetic acclimation to *T*. In contrast, Kattge and Knorr (2007) reported no consistent change in activation energy of V_{Cmax} with growth *T*. However, these two reviews used different parameterizations of the *T* dependence of V_{Cmax}, and Kattge and Knorr (2007) did find consistent shifts in the apparent optimum *T* of V_{Cmax} with growth *T*. An optimum *T* of V_{Cmax} of less than 40 ºC probably actually reflects deactivation of RuBPCO at high *T* (Sage and Kubien 2007), as the purified enzyme has an optimum *T* of at least 40 ºC. In *B. oleracea* and *A. thaliana*, the response of V_{Cmax} to *T* was exponential up to 35 ºC, indicating that deactivation of RuBPCO probably did not occur. While some have felt that the kinetic characteristics of RuBPCO should be uniform among species because of the conservative properties of the enzyme in higher plants (*e.g.* Bernacchi *et al.* 2001, but see Galmes *et al.* 2005), Yamori *et al.* (2005) argued that multiple forms of RuBPCO may coexist, and that the proportion of the different forms could shift the *T* dependency. Certainly a wide range of activation energies of V_{Cmax} based on gas exchange data have been observed in different species (Medlyn *et al.* 2002a, Kattge and Knorr 2007), although some of this variation could possibly result from estimating V_{Cmax} based on C_i rather than C_c. It is also possible that minor effects of *T* on the activation status of RuBPCO would appear as changes in activation energy. The activation energy found here in *A. thaliana*, 64–69 kJ mol^{-1}, is very similar to that in tobacco (65 kJ mol^{-1}) (Bernacchi *et al.* 2001) and spinach (70 kJ mol^{-1}) (Yamori *et al.* 2006b). The lower activation energies of V_{Cmax} at low growth *T*s observed here in *B. oleracea*, whether estimated from C_i or C_c, is same as the pattern summarized by Hikosaka *et al.* (2006). Medlyn *et al.* (2002b) found seasonal variation in the activation energy of V_{Cmax}, but no clear relationship with *T*. In *B. oleracea*, the shift in the optimum *T* of P_N measured at air levels of CO_2 with change in growth *T* was primarily caused by changes in the activation energy of V_{Cmax}.

There have only been a few studies which have reported the *T* dependence of g'_m, and only one report that examined acclimation to *T* of g'_m. Bernacchi *et al.* (2002) found an optimum *T* near 35 ºC for g'_m in tobacco, while Warren and Dryer (2006) reported that g'_m doubled from 10 to 20 ºC and then was independent of *T* above 20 ºC in an oak species. Yamori *et al.* (2006a) found a response curve with an optimum *T*, similar to that found in tobacco, when spinach had been grown at low *T*, but a flat-topped curve similar to that in oak, when spinach was grown at high *T*. My data obtained using the constant J fluorescence method indicated a linear increase in g'_m with *T* in *B. oleracea*, with a steeper response in plants grown at higher *T*. In contrast, g'_m in *A. thaliana* did not change significantly with either measurement or growth *T*.

However, g'_m estimated using the variable J method was much lower at 10 and 15 ºC in both *A. thaliana* and *B. oleracea*, resulting in flat-topped curves similar to those in oak (Fig. 2). Such low g'_m values would require very high V_{Cmax} values at those low *T*s to support the observed P_N at low C_c. The required high V_{Cmax} values at 10 and 15 ºC would have resulted in activation energies of V_{Cmax} of about 25 or less kJ mol^{-1}, which is less than half the value obtained with purified RuBPCO. It seems more likely that g'_m estimated by the variable J method at low *T* was incorrect, possibly because of alternative sinks for electrons at low *T*s (Harley *et al.* 1992). The sometimes large discrepancy between g'_m estimated from fluorescence signals obtained from the upper and lower surfaces also decreases confidence in g'_m estimated with the variable J method. Lichtenthaler *et al.* (2005) provided other examples where fluorescence signals from upper and lower leaf surfaces would lead to different conclusions about limitations to photosynthesis.

While Yarmori *et al.* (2006a) found that the change in g'_m with growth *T* in spinach contributed substantially to the observed shift in the optimum *T* of P_N, the changes in g'_m with growth *T* in *B. oleracea* did not affect the optimum *T* of P_N. The value of g'_m for *A. thaliana* I obtained was about three times higher than that reported at 25 ºC by Flexas *et al.* (2007b), but my plants were grown at much higher irradiance, and also had correspondingly higher P_N. Although g'_m does not always scale with P_N (Warren and Adams 2006), it often does (*e.g.* Loreto *et al.* 2002), and did so in *A. thaliana* with respect to leaf aging (Flexas *et al.* 2007b). My values of g'_m for both *A. thaliana* and *B. oleracea* are similar to those of other herbaceous annuals with high P_N summarized by Warren and Adams (2006).

In summary, in *A. thaliana* the only evidence of acclimation of P_N to *T* was the decrease in V_{Cmax} and J_{max} with growth at the highest *T*. In contrast, *B. oleracea* also had large changes in the *T* dependencies of V_{Cmax}, J_{max}, and g'_m and shifts in the optimum T of P_N. Thus *B. oleracea* has a much larger capacity for acclimation of photosynthesis to moderate changes in growth T than *A. thaliana*.

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