Rubisco catalytic properties optimized for present and future climatic conditions

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Because of its catalytic inefficiencies, Rubisco is the most obvious target for improvement to enhance the photosynthetic capacity of plants. Two hypotheses are tested in the present work: (1) existing Rubiscos have optimal kinetic properties to maximize photosynthetic carbon assimilation in existing higher plants; (2) current knowledge allows proposal of changes to kinetic properties to make Rubiscos more suited to changed conditions in chloroplasts that are likely to occur with climate change. The catalytic mechanism of Rubisco results in higher catalytic rates of carboxylation being associated with decreased affinity for CO2, so that selection for different environments involves a trade-off between these two properties. The simulations performed in this study confirm that the optimality of Rubisco kinetics depends on the species and the environmental conditions. In particular, environmental drivers affecting the CO2 availability for carboxylation (Ct) or directly shifting the photosynthetic limitations between Rubisco and RuBP regeneration determine to what extent Rubisco kinetics are optimally suited to maximize CO2 assimilation rate. In general, modeled values for optimal kinetic reflect the predominant environmental conditions currently encountered by the species in the field. Under future climatic conditions, photosynthetic CO2 assimilation will be limited by RuBP-regeneration, especially in the absence of water stress, the largest rise in [CO2] and the lowest increases in temperature. Under these conditions, the model predicts that optimal Rubisco should have high S/L and low Kc.

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1. Introduction

The enzyme Rubisco catalyses the assimilation of CO2 by the carboxylation of RuBP in the Calvin Cycle, and is therefore the most obvious target to improve the photosynthetic capacity of crops. Rubisco displays catalytic inefficiencies, including slow catalysis and imperfect discrimination between CO2 and O2 [1]. These inefficiencies not only limit the rate of CO2 fixation, but also compromise the capacity of crops to use resources optimally, particularly water and nitrogen [1,2]. In principle, overcoming these limitations could be successfully accomplished by molecular interventions in the genes coding for both the large (LSu) and the small (SSu) subunits of Rubisco [1,3–5]. Among these interventions, replacement of crop Rubiscos by other versions of the enzyme with better catalytic performance has recently been tested and provides a powerful and promising approach [6–8]. However, the success – in terms of photosynthetic improvement – of the Rubisco replacement strategy will depend on the discovery of more efficient and compatible versions of the enzyme. As an example, Ref. [9] illustrated how Rubiscos from red algae would perform better than tobacco native Rubisco if they could be transferred into the chloroplast of tobacco. A number of surveys have reported substantial variability among species in the kinetic parameters of Rubisco, the relative specificity for CO2/O2 (S), the Michaelis–Menten constants for CO2 (Kc) and O2 (Ko), and the maximum turnover of carboxylation (Kc) [5,10–15]. Among the potential forces driving evolution of Rubisco catalysis, the availability of CO2 for ribulose-1,5-bisphosphate (RuBP) carboxylation has been proposed as a major factor, explaining some of the differences among distant phylogenetic groups, between C3 and C4 species and among C3
plants [12,16,17]. Thus, those environments where plants have evolved under high temperatures and low soil water availability should be prioritized in the search for better versions of the enzyme [18]. Under these conditions, the CO2/O2 concentration at the site of carboxylation is decreased due to lower leaf conductances in response to water scarcity and lower CO2/O2 solubility ratios as temperature increases [19]. It has been demonstrated that Rubisco has evolved to higher $S_{c/o}$ under these conditions, reducing RuBP oxygenation and favoring the carboxylation reaction [13]. Nevertheless, comparisons of the scarce data on Rubisco kinetics – particularly data describing the full set of kinetic parameters on the same species – indicate a strong, negative correlation between $S_{c/o}$ and $k_{cat}^c$. Therefore, the suggestion that Rubiscos with high $S_{c/o}$ from extreme environments would allow higher CO2 assimilation rates when transferred into the chloroplast of a crop plant grown under non-stressed conditions, and perhaps even in dense canopies where light limits photosynthesis, is doubtful. The maximum leaf conductances typically measured under optimal growth conditions provide maximum CO2 availability at the site of carboxylation [2]. Under these conditions, a Rubisco with a higher $k_{cat}$, although at the expenses of a lower $S_{c/o}$, may provide the higher benefits [20,21].

The significant correlations between $k_{cat}$ and $S_{c/o}$ and $K_o$ – have led to the opinion that Rubisco is nearly perfectly adapted for differing CO2/O2 concentrations and thermal conditions in the chloroplast environment [14,20,22]. Structural and mechanistic constraints have not allowed the development – either naturally or artificially – of the perfect Rubisco with increased specificity to CO2 and a high rate of carboxylation. Natural evolution has resulted instead in Rubiscos in which there is a compromise between CO2/O2 affinities and the maximum rate of catalytic turnover dependent on the habitat and climate. This tradeoff is evident from a close inspection of published data which shows a significant scatter in Rubisco kinetics values and consequently in carboxylase catalytic efficiency ($k_{cat}^c/K_o$) [5,14,22]. Much less is known about the oxygenase kinetic parameters, although some correlation between oxygenase and carboxylase catalytic constants has been observed [23].

Maximum agricultural yields are obtained by growing crops with non-limiting resources by extensive irrigation and fertilization practices. However, the environmental footprint of intensive agriculture and the predictions for higher global temperatures and lower water availability for most current cropping areas demand novel solutions toward a more sustainable agriculture. It is widely believed that a more efficient Rubisco would provide not only more photoassimilates for the plant growth, but importantly do this at a lower cost of water and nutrients [24,25]. However, given the tradeoff between $k_{cat}^c$ and $S_{c/o}$, it is not immediately evident what more efficient means in environmental and physiological contexts. Within this context, the present paper aims at the following: (i) to model how temperature and O2 and CO2 concentration affect Rubisco kinetics; (ii) to test whether current Rubiscos are optimally suited for the present conditions in the chloroplast of higher plants; (iii) to estimate the potential impact of climate change on the optimality of Rubisco kinetics and to identify targets of improvement. We argue that it is necessary to consider the tradeoff between specific activity and specificity to define rigorous criteria for engineering plants with more efficient Rubisco.

### 2. Methods and theory

#### 2.1. Optimal Rubisco

According to the biochemical model of leaf photosynthesis [26], the net assimilation rate ($A$) is determined by the minimum of the RuBP-saturated ($A_c$) and RuBP-limited ($A_j$) CO2 assimilation rates:

$$A = \min(A_c, A_j),$$  

$$A_c = \frac{V_{cmax} (C_c - \Gamma_c^-)}{C_c + K_c (1 + O/K_o)} - R_d$$  

$$A_j = \frac{(C_j - \Gamma_j^+)/4}{C_j - 2\Gamma_j^+} - R_d$$  

where $V_{cmax}$ is the maximal carboxylation rate, $C_c$ and $O$ are the concentration of CO2 and O2, respectively. $K_o$ is the Michaelis–Menten constant for CO2 and $K_c$ is that for O2. $\Gamma_c$ is the CO2 compensation point in the absence of mitochondrial respiration. $J$ is the CO2-saturated electron transport rate of the thylakoid reactions which ultimately supply the necessary energy in the form of ATP and NADPH for the regeneration of RuBP, and $R_d$ is the mitochondrial respiration in the light. The response of $J$ to the photosynthetic photon flux density (PPFD) was calculated from the non-rectangular hyperbola proposed by Bernacchi et al. [27].

In Eq. (2), $V_{cmax}$ was obtained from in vitro values as:

$$V_{cmax} = k_{cat} E \cdot Act$$

$k_{cat}$ is the Rubisco maximum turnover rate of carboxylation, $E$ is the total quantity of catalytic sites and $Act$ is the percentage of activation of Rubisco sites, i.e., its carbamylation state.

$$\Gamma_c^+$$ was obtained from the Rubisco specificity factor, $S_{c/o}$, as:

$$\Gamma_c^+ = 0.50 \frac{S_{c/o}}{K_c}$$

Because the Rubisco kinetic parameters are interrelated [5,28], we can express Eqs. (2) and (3) as determined by a unique kinetic parameter, similar to the approach followed by Savir et al. [14].

$k_{cat}^c$ is the most reported parameter, probably due to its relative ease of measurement. Thus, Eqs. (2) and (3) were reformulated by substituting $\Gamma_c^+$, $K_c$ and $K_o$ by the statistical functions relating them to $k_{cat}^c$. To obtain the relationships of $\Gamma_c^+$, $K_c$ and $K_o$ vs. $k_{cat}^c$ at 25 °C we used the data compiled in Savir et al. [14], excluding the photosynthetic bacteria. The regression coefficients were highly significant ($P < 0.001$), with $r^2 > 0.75$ (Table 1S). Because the different kinetic parameters present distinct sensitivities to temperature [29], the functions relating $k_{cat}^c$ with the remaining parameters are dependent on temperature. Hence, to interrelate the Rubisco kinetic parameters at temperatures other than 25 °C, values of $k_{cat}^c$, $\Gamma_c^+$, $K_c$ and $K_o$ were converted to the desired temperature using the equations described in Bernacchi et al. [29] for tobacco with the in vitro species–specific data measured at 25 °C as the reference (i.e., the scaling constant $c$). Thereafter, regression analyses were again run between $k_{cat}^c$ and $\Gamma_c^+$, $K_c$ and $K_o$ (Table 1S). The analysis therefore assumes that the Rubisco kinetic parameters of the species included in the present study presented the same temperature dependency as that for Rubisco from Nicotiana tabacum, and that the equations hold for the whole range of temperatures from 15 °C to 38 °C considered in the present study [30].

#### 2.2. Optimization of Rubisco kinetics at varying temperature, and CO2 and O2 concentration

A first aim of the study was to model how temperature and [CO2] and [O2] affect Rubisco kinetics. For this, the optimal value for $k_{cat}^c$ (i.e., the one maximizing the net CO2 assimilation rate) was modeled at varying $C_c$ and given values for $J$, $R_d$, $E$ and $Act$ by solving Eqs. (1)–(3) expressed in terms of $k_{cat}^c$ as the unique parameter, using Mathematica 9 software (Wolfram Research, Champaign, IL, USA). This analysis was performed at three different temperatures (15, 25
and 35 °C and [O₂] (1000, 170,000 and 210,000 μmol mol⁻¹). The optimal values for S_C/O₂ (Γ⁺), K_c and K_C were thereafter obtained from the functions relating them with k_cat, at the desired temperature (Table 1S).

2.3. Optimization of Rubisco kinetics under present conditions

The second aim of the study was to test whether current Rubiscos are optimally suited for the present environmental conditions. By using the same equations, the net CO₂ assimilation rate was calculated at a range of varying k_cat values for Limonium gibraltar, Nicotiana tabacum and Triticum aestivum, under different environmental conditions (25 °C-well watered, 25 °C-water stress, 38 °C-water watered and 38 °C-water stress). L. gibraltar was selected as being one of the most attractive species in terms of Rubisco traits due to its high specificity [13,31]. N. tabacum is the species where most of attempts to bioengineer Rubisco have been made [32], while T. aestivum is one of the most important crops worldwide supporting a significant part of the human caloric intake. Values for the model input parameters: C_c, J, R_d, E and Rubisco activation state under the different environmental conditions are shown in Table 1, and were taken from Galmés et al. (unpublished), Galmés et al. [33] and Perdomo et al. (unpublished), for L. gibraltar, N. tabacum and T. aestivum, respectively. The growth conditions were similar for the three original species, and the plants were acclimated (i.e. grown for at least one month) to the experimental conditions.

Table 1
Leaf photosynthetic parameters for Limonium gibraltar, Nicotiana tabacum and Triticum aestivum under different conditions of water availability (WW: well-watered; WS: water stress) and leaf temperature. Values for the net CO₂ assimilation rate (A), stomatal conductance (gₛ), mesophyll conductance (g_mE), CO₂ concentration in the chloroplast (C_c), rate of electron transport (J), mitochondrial respiration in the light (R_d), concentration of Rubisco sites (E) and Rubisco activation state. From Galmés et al. (unpublished), Galmés et al. [33] and Perdomo et al. (unpublished) for L. gibraltar, N. tabacum and T. aestivum, respectively. Photosynthetic photon flux density and oxygen concentration during measurements were constant at 1500 μmol m⁻² s⁻¹ and 210,000 μmol mol⁻¹, respectively. Growth conditions were similar for the three original species, and the plants were acclimated (i.e. grown for at least one month) to the experimental conditions.

<table>
<thead>
<tr>
<th></th>
<th>L. gibraltar</th>
<th>N. tabacum</th>
<th>T. aestivum</th>
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<tr>
<td></td>
<td>25 °C-WW</td>
<td>25 °C-WS</td>
<td>25 °C-WW</td>
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<tr>
<td>A (μmol m⁻² s⁻¹)</td>
<td>23.5</td>
<td>10.8</td>
<td>23.8</td>
</tr>
<tr>
<td>gₛ (mol m⁻² s⁻¹)</td>
<td>0.306</td>
<td>0.095</td>
<td>0.335</td>
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<tr>
<td>g_mE (mol m⁻² s⁻¹)</td>
<td>0.354</td>
<td>0.099</td>
<td>0.404</td>
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<tr>
<td>C_c (μmol mol⁻¹)</td>
<td>167.1</td>
<td>80.1</td>
<td>206.4</td>
</tr>
<tr>
<td>J (μmol m⁻² s⁻¹)</td>
<td>132.3</td>
<td>157.6</td>
<td>175.6</td>
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<tr>
<td>R_d (μmol m⁻² s⁻¹)</td>
<td>2.1</td>
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<td>1.2</td>
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<tr>
<td>E (μmol m⁻²)</td>
<td>64.0</td>
<td>81.7</td>
<td>75.5</td>
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<tr>
<td>Activation state (%)</td>
<td>75</td>
<td>65</td>
<td>77</td>
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Table 2
Current and optimal values of the net CO₂ assimilation rate (A) and in vitro Rubisco kinetics for Limonium gibraltar, Nicotiana tabacum and Triticum aestivum under different conditions of water availability (WW: well-watered; WS: water stress) and leaf temperature. Values for A were experimentally measured (same as in Table 1). Current values at 25 °C of the maximum turnover rate of carboxylation (K_c), the CO₂ compensation point in the absence of mitochondrial respiration (J⁺), and the Michaelis-Menten constants of Rubisco for CO₂ (K_C) and O₂ (K_O) were obtained from Galmés et al. (unpublished), Whitney et al. [5], and Savir et al. [14] for L. gibraltar, N. tabacum and T. aestivum, respectively. Values of in vitro Rubisco kinetics for N. tabacum at 38 °C were obtained after applying temperature equations from Bernacchi et al. [20], but using measured data at 25 °C as the reference.

<table>
<thead>
<tr>
<th></th>
<th>L. gibraltar</th>
<th>N. tabacum</th>
<th>T. aestivum</th>
</tr>
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<tbody>
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<td></td>
<td>25 °C-WW</td>
<td>25 °C-WS</td>
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<tr>
<td>Current values</td>
<td>23.5</td>
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<td>23.8</td>
</tr>
<tr>
<td>k_cat (s⁻¹)</td>
<td>2.5</td>
<td>3.2</td>
<td>4.2</td>
</tr>
<tr>
<td>Γ⁺ (mol mol⁻¹)</td>
<td>35.2</td>
<td>48.2</td>
<td>43.9</td>
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<tr>
<td>K_c (μmol mol⁻¹)</td>
<td>273</td>
<td>339</td>
<td>443</td>
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<tr>
<td>K_O (μmol mol⁻¹)</td>
<td>341,671</td>
<td>233,468</td>
<td>622,014</td>
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<td>Optimal values under A, limit</td>
<td>21.2</td>
<td>8.5</td>
<td>33.8</td>
</tr>
<tr>
<td>A (μmol m⁻² s⁻¹)</td>
<td>2.5</td>
<td>3.2</td>
<td>4.2</td>
</tr>
<tr>
<td>k_cat (s⁻¹)</td>
<td>3.3</td>
<td>1.9</td>
<td>3.6</td>
</tr>
<tr>
<td>Γ⁺ (mol mol⁻¹)</td>
<td>45.6</td>
<td>31.0</td>
<td>47.8</td>
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<tr>
<td>K_c (μmol mol⁻¹)</td>
<td>422</td>
<td>264</td>
<td>465</td>
</tr>
<tr>
<td>K_O (μmol mol⁻¹)</td>
<td>376,046</td>
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<td>385,614</td>
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<td>Optimal values under A, A co-limit</td>
<td>18.0</td>
<td>8.5</td>
<td>27.8</td>
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<tr>
<td>A (μmol m⁻² s⁻¹)</td>
<td>1.8</td>
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<td>k_cat (s⁻¹)</td>
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<td>K_c (μmol mol⁻¹)</td>
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<td>333,502</td>
<td>331,884</td>
</tr>
<tr>
<td>K_O (μmol mol⁻¹)</td>
<td>342,472</td>
<td>342,472</td>
<td>342,472</td>
</tr>
</tbody>
</table>

2.4. Optimization of Rubisco kinetics under future conditions

The third aim of the present study was to evaluate the potential effects of climate change on current optimization of Rubisco kinetics in wheat. For this, eight different scenarios of change were...
considered, each one representing a specific combination of the three main drivers of climate change: atmospheric CO₂ rise (550 or 800 μmol mol⁻¹), temperature increase (2–5 °C increase with respect to 25 °C) and water availability (well watered and moderate water stress). The intensities of change in atmospheric [CO₂] (Cₐ) and temperature were selected according to IPCC predictions [34]. Atmospheric [CO₂] is projected to continue rising to at least 550 ppm by 2050, and by 2100 the expected increase in air temperature is between 2 and 5 °C, depending on the region and the applied model [35].

The impact of each climate change scenario on the stomatal conductance (gs) was quantified in relation to the current gs measured in wheat under optimal conditions (Perdomo et al., unpublished) as follows: 20% and 50% decrease at Cₐ of 550 and 800 ppm, respectively [36], 1.5% increase per °C increase in air temperature according to Evans and von Caemmerer [37], and 50% decrease under moderate water stress according to Galmés et al. [38]. Thereafter, considering the leaf mesophyll conductance (gₘ) measured in the same experiment, a gₘ/gs ratio of 0.39 was obtained at 25 °C. We are aware that these values for the gₘ/gs ratio are lower than typical reported values for a range of species [39], but given the natural variability in this ratio we preferred to use values recently measured on wheat in our laboratory. The gₘ/gs ratio was changed at 27 °C (0.44) and 30 °C (0.52) assuming the response to temperature of gₘ published for tobacco in Walker et al. [40].

The concentration of Rubisco catalytic sites and its activation state were also taken from 25 °C-well watered plants (Perdomo et al., unpublished) and assumed to be constant for all climate change scenarios (Table 25). According to a recent review, at the expected increase in Cₐ, temperature and severity of water stress, the changes in Rubisco concentration and activation will be minor [41]. By using in vitro published data for wheat Rubisco at 25 °C [14], Vₐmax was calculated at 25 °C applying Eq. (4). A constant ratio Iₐmax/Vₐmax of 1.5 has been measured in wheat in our laboratory (Perdomo et al., unpublished), which matches very well with other estimates recently published in wheat [42]. Vₐmax and Iₐ max temperature responses were assumed to be similar to those obtained for tobacco by Walker et al. [40]. Oxygen concentration was taken as 210,000 μmol m⁻² s⁻¹ and PPFD as 1500 μmol m⁻² s⁻¹.

Finally, we ended up with two unknowns, A and Cₐ, and two equations, (2) and (3) for A and Fick’s law for Cₐ:

$$Cₐ = C₀ - \frac{A}{gs} - \frac{A}{gₘ}$$

The resultant quadratic equation for Rubisco-limited photosynthesis (2) was solved according to von Caemmerer and Evans [43] and Ether and Livingston [44], and that for RuBP-limited photosynthesis (3) solved according to Niinemets et al. [45].

All analyses were performed considering A to be first strictly limited by A₀, and then also including A₁ limitation.

### 3. Results and discussion

#### 3.1. Optimization of Rubisco kinetics at varying temperature, and CO₂ and O₂ concentration

Past surveys compiling data on Rubisco kinetics and specific activity are in agreement that the maximum turnover rate of carboxylation (kₐcat) and the affinity for CO₂ (i.e. the inverse of the Michaelis– Menten constant for CO₂, Kₐ) or the relative specificity for CO₂ and O₂ (Sₒ/o) are inversely related [5,14,22,28,46,47]. This suggests constant values for the carboxylase catalytic efficiency (kₐcat/Kₐ) at given temperature and substrate concentration [48], and implies that the rate of carboxylation cannot be improved without losing affinity for CO₂ [5,20,49]. A direct consequence of limited variability in kₐcat/Kₐ is that each particular Rubisco has been forced to evolve toward optimizing either the velocity or the affinity to CO₂. Although conclusive proofs about the environmental factors driving the evolution of Rubisco have not been provided, there is some evidence that the concentration of the two gaseous substrates, CO₂ and O₂, and long-term temperature environment are playing a decisive role [13,17,22,50]. It is therefore relevant to explore how these key environmental factors modulate Rubisco performance.

We define optimal Rubisco as Rubisco having a combination of kinetic traits yielding the maximum photosynthesis under given environmental conditions. According to the Cₐ photosynthesis model [26], the rate of CO₂ assimilation (A) is the minimum of the ribulose-1,5-bisphosphate (RuBP)-saturated rate of photosynthesis (Aₑ) and the RuBP-limited rate (Aᵣ). Consequently, it is important to consider both limitations in assessing the kinetic values for an optimal Rubisco.

At a constant concentration of Rubisco active sites (24 μmol m⁻²), and strictly considering the Aᵣ-limited rate of photosynthesis, increasing the concentration of CO₂ in the chloroplast stroma (Cₐ) shifts optimality toward increased kₐcat, and decreased Sₒ/o (Fig. 1), in agreement with previous analyses [49]. This fact explains the benefits of the catalytic modifications in Cₐ Ruboscis under saturating [CO₂]. In particular, on average greater kₐcat values in Cₐ than in Cₐ plants [8,51]. The dependency of optimum kₐcat on Cₐ is non-linear, being greater at lower Cₐ, and decreasing at higher Cₐ (Fig. 1). The inclusion of Aᵣ-limitation in the analysis changes the response of the optimum kₐcat on Cₐ. In the initial phase, at the Aᵣ-limited region, optimum kₐcat increases with Cₐ until a certain threshold at which Aₑ = Aᵣ. When the threshold Cₐ is exceeded, A becomes Aᵣ-limited, after which the optimum kₐcat decreases with increasing Cₐ (Fig. 1). We note that the threshold Cₐ depends not only on Rubisco kinetics, but also on light intensity that modifies the rate of RuBP regeneration and on temperature effects on the capacity for photosynthetic electron transport (for explicit derivation of the condition of co-limitation see e.g. [52]).

Due to the tradeoff between kₐcat and Sₒ/o, the response of optimum Sₒ/o on Cₐ was opposite to that for optimum kₐcat (Fig. 1). For the Aᵣ-limited rate of photosynthesis, Sₒ/o for an optimal Rubisco increased even at high Cₐ. This fact explains the predicted beneficial effects of replacing wheat Rubisco by that from *L. gibertii* that has a higher Sₒ/o [53].

Regardless of whether A is limited by Rubisco or RuBP regeneration, the relationship between the optimal kₐcat/Kₐ and Cₐ exhibited a biphasic response. Optimal kₐcat/Kₐ increased with Cₐ until a maximum value, after which further increases in Cₐ resulted in decreases in kₐcat/Kₐ (Fig. 1). Remarkably, the Cₐ value at which the maximum value of optimal kₐcat/Kₐ was attained, ca. 100 μmol mol⁻¹ at 15 °C, 150 μmol mol⁻¹ at 25 °C and 200 μmol mol⁻¹ at 35 °C, roughly coincides with the values typically reported for Cₐ in Cₐ species under non-stressful well-watered conditions and current ambient atmospheric CO₂ concentrations. The reported Cₐ values range from 200 μmol mol⁻¹ in crops and herbaceous plants [37,54–56] to less than 100 μmol mol⁻¹ in sycamore leaves with high leaf mass per area [45,56–60].

Rubisco kinetic parameters are all very sensitive to temperature [13,61–63]. However, the relative effect of a given change in temperature is different for each kinetic parameter, due to their differential temperature dependence [29,64]. This can be observed in Fig. 1, where the proportional effect of altered temperature was different in the response of optimal kₐcat, Sₒ/o and kₐcat/Kₐ to varying Cₐ (Fig. 1). In the analysis considering Aᵣ limitation (Fig. 1 right-side panels), an increase in temperature increased the threshold value of Cₐ where maximum and minimum values for optimal kₐcat and Sₒ/o are obtained. This is because temperature affects the Cₐ at which
the transition from Rubisco to RuBP regeneration limitation occurs [64–66].

Changes in the concentration of O2 also conditioned the above described responses of optimal $k_{cat}^{c}$ and $S_{i/o}$ to varying C$_{i}$ (Fig. 1S). As hypothesized [67], an atmosphere with very little molecular oxygen corresponding to the geological past when Rubisco evolved more than 2.5 billion years ago, would favor a Rubisco with increased $k_{cat}^{c}$, and decreased $S_{i/o}$, but only in the case when A$_{i}$ limits A. Under low [O2] and for A$_{i}$-limited A, high C$_{i}$ would demand Rubiscos with lower $k_{cat}^{c}$ and higher $S_{i/o}$ (Fig. 1S). According to the biochemical model of photosynthesis [26], under A$_{i}$ limitation, the unique kinetic parameter of Rubisco directly determining A is $S_{i/o}$ (or $I^*$). Therefore, the demanded lower $k_{cat}^{c}$ is simply an indirect effect of the tradeoff between $k_{cat}^{c}$ and $S_{i/o}$.

3.2. Rubisco is not optimal in current environmental conditions of C$_{3}$ plants

Empirical data of leaf gas-exchange, chlorophyll fluorescence and Rubisco biochemistry were compiled for the perennial semi-shrub *L. gibertii* adapted to stressful environments in shallow, salty soils in the Mediterranean, annual herb *N. tabacum* (tobacco) and annual grass *T. aestivum* (wheat) from experiments performed under similar environmental conditions (Table 1). These data were used to model A over a range of values of $k_{cat}^{c}$, to test whether Rubisco kinetics of these three species were optimal under the characteristic environmental conditions encountered in the field (Fig. 2).

First, we considered the predictions under well-watered non-stressed conditions at 25 °C and assumed RubBP-saturated rate of A. Under these conditions, the modeled optimal Rubisco had higher $k_{cat}^{c}$ and $K_{c}$ than reported in vitro values for three species (Table 2). Deviations from the actual and the modeled optimum values of $k_{cat}^{c}$ under these conditions were higher in *L. gibertii* and wheat, and less in tobacco (Fig. 2). Water stress, by reducing C$_{i}$, decreases the optimal values of $k_{cat}^{c}$ (Fig. 2), in agreement with the idea that increasing $S_{i/o}$ would be particularly beneficial under semi-arid and arid conditions characterized by most severe limitation of photosynthesis by water availability [15,52]. Under water stress at 25 °C, the modeled optimal Rubisco had a lower $k_{cat}^{c}$ and $K_{c}$ than the reported in vitro values for *L. gibertii* and tobacco, while modeled and in vitro values perfectly matched in wheat (Table 2). In wheat, increasing the leaf temperature to 38 °C augmented the modeled optimal $k_{cat}^{c}$, but the correspondence with temperature-corrected in vitro values was maintained. Subsequent application of water

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**Fig. 1.** Optimal values for the maximum turnover rate of carboxylation ($k_{cat}^{c}$), specificity factor ($S_{i/o}$) and carboxylation catalytic efficiency ($k_{cat}^{c}/K_{c}$) at a range of CO$_2$ concentrations in the chloroplast (C$_{i}$) at 15 °C (empty circles), 25 °C (filled circles) and 35 °C (empty triangles). The photosynthetic rate was considered to be limited strictly by Rubisco (A$_{i}$ limited, left graphs) or co-limited by Rubisco and RuBP-regeneration capacity (A$_{i}$, A$_{c}$ co-limited, right graphs). The rates of electron transport (J) were 42, 74 and 106 µmol m$^{-2}$ s$^{-1}$ at 15 °C, 25 °C and 35 °C, respectively. The rates of mitochondrial respiration in the light (R$_{o}$) were 0.5, 1.0 and 1.8 µmol m$^{-2}$ s$^{-1}$ at 15 °C, 25 °C and 35 °C, respectively. The concentration of Rubisco catalytic sites (30 µmol m$^{-2}$) and its activation state (80%) were considered invariable at the three temperatures.
Fig. 2. Net CO₂ assimilation rate (A) at a range of values for the maximum turnover rate of carboxylation ($k_{cat}^c$) for *Limonium gilbertii*, *Nicotiana tabacum* and *Triticum aestivum*, under non-stress conditions: 25 °C-well-watered (filled circles), 25 °C-water stress (empty circles), 38 °C-well-watered (filled triangles) and 38 °C-water stress (empty triangles). The photosynthetic rate was considered to be limited strictly by Rubisco ($A_i$, limited, left graphs) or co-limited by Rubisco and RuBP-regeneration capacity ($A_c$, $A_i$ co-limited, right graphs). The dotted line indicates the actual $k_{cat}^c$ value for the three species. The input values of the model are shown in Table 1. The equations describing the correlation among Rubisco kinetic parameters at the different temperatures are shown in Table 15.

stress to wheat grown at 38 °C decreased the optimal $k_{cat}^c$ to values slightly lower than the in vitro ones (Table 2). Overall, evaluating the departure from optimality of all characteristics of Rubisco as the average deviation from optimality for individual traits, $k_{cat}^c$, $S_{c/o}$ ($J^*$) and $K_c$ modeled under $A_i$-limited photosynthesis, Rubiscos of *Limonium* and wheat were better suited for conditions typically measured under water stress, while tobacco Rubisco lost optimality under water stress (Table 3). These results may be a consequence of the enzyme evolution under the predominant environmental conditions encountered by these species in the field. The rocky, saline and hot areas under Mediterranean climate inhabited by *Limonium* species necessitate a strict control of water loss by stomatal closure, thereby restricting the CO₂ availability for carboxylation [57]. On the other hand, wild wheat relatives and the first domesticated varieties of wheat were drought and salt tolerant [68,69]. These results corroborate the results of a past simulation study showing that current Rubisco kinetics of many C₃ plants are better suited for low [CO₂] [49].

In well-watered plants grown at 25 °C and at moderately high light of 1500 μmol m⁻² s⁻¹, $A$ was limited by the rate of RuBP-regeneration ($A_i$) in all three species (Table 2). Under these conditions, optimum $k_{cat}^c$ is determined at the $A_i$ to $A_i$ co-limitation point. At this point, the values of optimum $k_{cat}^c$ were lower than those modeled strictly under $A_i$-limitation (Fig. 2). The values of optimum $k_{cat}^c$, $K_c$ and $J^*$ under $A_c$, $A_i$ co-limitation were lower than in vitro reported values (Table 2). Under water stress at 25 °C, the optimum $k_{cat}^c$ corresponded to the $A_c$-limited rate in *Limonium* and tobacco, but still to $A_c$, $A_i$-co-limited rate in wheat, with slight increase in the optimum values compared with the well-watered treatment (Fig. 2, Table 2). By increasing the temperature to 38 °C in wheat, $A$ became $A_i$ limited and optimal $k_{cat}^c$ increased as explained above (Fig. 2). The overall optimality of Rubisco kinetics after considering $A_i$-limitation was improved only in *Limonium* at 25 °C and well-watered conditions (Table 3).

This analysis and the simulation by Zhu et al. [49] focusing on C₃ plants reveal that Rubisco kinetics are far from being
optimal, and that there is room for improvement. Our results highlight that the optimality of Rubisco kinetics depends on the species and the environmental conditions. In fact, due to the inherent tradeoff between $S_{\text{CP}}$ and $K_{\text{cat}}$, being optimal under some circumstances, such as water limitations, results in non-optimality in well-watered conditions, especially if there is a cross-over to RubP-limited photosynthesis. As this analysis further demonstrates that the occurrence of RubP-limitation itself is an important factor altering the Rubisco optimality. In fact, plants are exposed to varying light conditions during the day and only uppermost leaves in the canopy are exposed to full sunlight [45,70,71], implying that a large fraction of leaves in the canopy is RubP-limited at any moment of time and all leaves are RubP-limited at some moment during the day. In a typical canopy, light distribution is heterogeneous and $V_{\text{max}}$ and $J_{\text{max}}$ are distributed accordingly. However, the ratio of $V_{\text{max}}$ to $J_{\text{max}}$ does not differ much between sites [72]. This drove to Chen et al. [73] to propose the coordination theory in which nitrogen inverted in the different photosynthetic pools of the leaves produce a co-limitation by $A_c$ and $A_i$ on a daily average. Taken all this, optimization of Rubisco needs consider RubP-limited photosynthesis as well.

### 3.3. Optimality of Rubisco kinetics will be affected by climate change

The optimality of Rubisco kinetics was tested under different scenarios of climate change, including varying conditions of atmospheric CO$_2$, temperature and water availability, and compared to modeled optimality under present conditions. Based on assumptions of how the environmental changes will impact stomatal ($g_s$) and mesophyll ($g_m$) conductances to CO$_2$, dark respiration rate ($K_d$), photosynthetic electron transport rate ($J$) and Rubisco traits (see Section 2), a value for $C_c$ corresponding to given set of environmental drivers (ambient CO$_2$ concentration, light intensity, temperature) was obtained.

Irrespective of whether photosynthesis was RubP-saturated or -limited, the overall optimality of Rubisco kinetics ($K_{\text{cat}}$, $I^*$ and $K_c$) increased in relation to current performance, in three of the eight scenarios of change, decreased in three and did not change significantly in two (Table 4). Under moderately increased [CO$_2$] up to 550 µmol mol$^{-1}$, shift of Rubisco performance toward increased optimality is expected under those scenarios with decreased water availability, regardless of the increase in temperature

### Table 3

Rubisco kinetics optimality in *Limonium gibraltar*, *Nicotiana tabacum* and *Triticum aestivum* under different conditions of water availability (WW: well-watered; WS: water stress) and leaf temperature. Rubisco kinetics optimality was calculated as the average of the optimality for the maximum turnover rate of carboxylation ($K_{\text{cat}}$), the CO$_2$ compensation point in the absence of mitochondrial respiration ($I^*$) and the Michaelis–Menten constant of Rubisco for CO$_2$ ($K_c$). The photosynthetic rate was considered to be limited strictly by Rubisco ($A_c$ limitation) or co-limited by Rubisco and RubP-regeneration capacity ($A_i$, $A_c$ co-limitation). The input values of the model are shown in Table 1. The equations describing the correlation among Rubisco kinetic parameters at the different temperatures are shown in Table 15.

<table>
<thead>
<tr>
<th></th>
<th>$A_c$ limitation</th>
<th>$A_i$, $A_c$ co-limitation</th>
</tr>
</thead>
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<tr>
<td><em>L. gibraltar</em></td>
<td></td>
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<tr>
<td>25 C-WW</td>
<td>37.9</td>
<td>17.3</td>
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<tr>
<td>25 C-WS</td>
<td>13.6</td>
<td>13.6</td>
</tr>
<tr>
<td><em>N. tabacum</em></td>
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<td></td>
</tr>
<tr>
<td>25 C-WW</td>
<td>16.7</td>
<td>34.6</td>
</tr>
<tr>
<td>25 C-WS</td>
<td>24.1</td>
<td>24.1</td>
</tr>
<tr>
<td><em>T. aestivum</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 C-WW</td>
<td>14.4</td>
<td>40.4</td>
</tr>
<tr>
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<td>12.5</td>
</tr>
<tr>
<td>38 C-WS</td>
<td>17.0</td>
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</tbody>
</table>

### Table 4

Rubisco kinetics optimality in *Triticum aestivum* under different scenarios of climate change, at varying atmospheric CO$_2$ concentration ($C_c$), leaf temperature ($T_{\text{leaf}}$) and water status (WW: well-watered; WS: water stress). The photosynthetic rate was considered to be limited strictly by Rubisco ($A_c$ limitation) or co-limited by Rubisco and RubP-regeneration capacity ($A_i$, $A_c$ co-limitation). The input values of the model are shown in Table 2. The equations describing the correlation among Rubisco kinetic parameters at the different temperatures are shown in Table 15.

<table>
<thead>
<tr>
<th>Climate change scenario</th>
<th>$C_c$</th>
<th>$T_{\text{leaf}}$</th>
<th>Water status</th>
<th>$A_c$ limitation</th>
<th>$A_i$, $A_c$ co-limitation</th>
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<tr>
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<td>550</td>
<td>27</td>
<td>WW</td>
<td>30.1</td>
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<tr>
<td>2</td>
<td>550</td>
<td>27</td>
<td>WS</td>
<td>14.0</td>
<td>34.5</td>
</tr>
<tr>
<td>3</td>
<td>800</td>
<td>27</td>
<td>WW</td>
<td>44.6</td>
<td>55.5</td>
</tr>
<tr>
<td>4</td>
<td>800</td>
<td>27</td>
<td>WS</td>
<td>24.9</td>
<td>44.1</td>
</tr>
<tr>
<td>5</td>
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<td>WS</td>
<td>17.3</td>
<td>40.4</td>
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</table>

![Fig. 3](image-url) The relationship between the predicted change in optimal Rubisco in *T. aestivum* under the different scenarios of climate change (indicated in Table 4) and the chloroplastic CO$_2$ concentration ($C_c$) estimated for the same scenarios. Values of the predicted change in optimal Rubisco are normalized to the current values (set at 1), <1 means improvement of Rubisco optimality, >1 means decrement of Rubisco optimality. The photosynthetic rate was considered to be limited strictly by Rubisco ($A_c$ limitation, filled circles and solid line) or co-limited by Rubisco and RubP-regeneration capacity ($A_i$, $A_c$ co-limitation, empty circles and dashed line).
At the calculated \(C_v\) values, photosynthesis is limited by RuBP-regeneration (\(A_v\)) for all scenarios of change, especially in those scenarios with no water stress, highest rise in the \([CO_2]\), and lowest increase in leaf temperature (Table 2S). In general, under \(A_c\), \(A_v\) co-limitation, values of optimal \(k_{cat}^c\) increased in all cases, as compared to current values, except the scenario predicting no increase in water stress, 800 \(\mu\)mol \(CO_2\) mol\(^{-1}\) and 2°C increase in leaf temperature. Noticeably, this is the scenario with the highest predicted increase in \(C_v\) (Table 2S). Regardless of the increase in optimal \(k_{cat}^c\), relative to present situation, in absolute terms, optimal \(k_{cat}^c\) under all scenarios is lower than in \textit{vitro} reported data (Table 2S). In consequence, under future climate, optimal Rubisco for \(A_v\)-limited \(A\) should have a higher \(S_{ci}\). This finding should be considered in next attempts to engineer Rubisco of crop plants for a higher photosynthetic capacity. Previous simulations ignoring changes in environmental conditions showed that a decrease, not an increase in \(S_{ci}\) would enhance \(CO_2\) assimilation when photosynthesis was \(A_v\)-limited, but already pointed out that increasing \(S_{ci}\) would maximize carbon gain under \(A_v\)-limitation [22,52]. At the crop level, this trend for light-limited photosynthesis under future climate is supported by experimental evidence showing that canopies become denser with plants having higher leaf area indices at higher \(CO_2\) [74,75]. This may be even more important in wild plants growing under strong light competition. Accordingly, interspecific differences in \(S_{ci}\) [13] may strongly modify the competitive potential of different species and thereby alter species dominance and range in future. As demonstrated, the situation can be dramatically different when the severity of drought increases, as expected for a series of ecosystems, in particular in Mediterranean and other semi-arid ecosystems [76]. Such important aspects, including species differences in \(C_s\) [56], are not currently considered in modeling plant responses to future conditions. Of equal importance is that future modeling approaches should consider the effects of \(K_{o}\) for RuBP under dynamic conditions when light changes rapidly within seconds to minutes, leading to significant changes in the pool size of RuBP [77].

Optimization of Rubisco kinetics largely depends on the ratio between the maximum capacity for carboxylation (\(V_{max}\)) and the capacity for regeneration of RuBP (\(J_{max}\)). This is because the \(V_{max}/J_{max}\) ratio together with light intensity, determines the \(C_v\) value at which the transition between the \(A_c\) and \(A_v\)-limited rates occurs. In fact, it has been suggested that, ideally, a crop plant should express a high \(k_{cat}^c\)-Rubisco in the upper canopy leaves, exposed to full sunlight, higher temperature and water deficits (therefore \(A_c\)-limited), and a high \(S_{ci}\)-Rubisco in the shaded lower canopy leaves (\(A_v\)-limited) [20,49]. Some reports demonstrate that the expected increments in the photosynthetic rate due to higher \([CO_2]\) may be counteracted by limited nitrogen availability [78,79]. Actually, under elevated \(CO_2\), redistribution of nitrogen allocation from Rubisco toward RuBP-regeneration has been documented with clear effects on \(V_{max}/J_{max}\) ratio [36,80]. In view of reports compiled in a recent review [44], we have considered constant concentration of Rubisco active sites. However, changes in the activity of Rubisco Activase and in the concentration of Rubisco inhibitors would directly impact on the concentration of Rubisco active sites. Hence, the observed decreases in the Rubisco activation state at elevated \([CO_2]\) [81], the decreased activity of Rubisco activase under high temperatures [82], or the increased concentration of tight-binding inhibitors reported in several species under severe drought [25,83] would lead to lower \(V_{max}\). Thus, a decrease in \(V_{max}/J_{max}\) could shift the limitation toward \(A_c\), and therefore, increase the relevancy of \(k_{cat}^c\) over \(S_{ci}\). In fact, this point is equally valid for acclimation to altered temperatures, where cross-over \(C_v\) concentration might change due to different temperature relationships of \(J_{max}\) and \(V_{max}\) (Fig. 1) [84,85].

### 4. Concluding remarks

Previous analyses of Rubisco optimization suggested that the kinetic parameters are nearly perfectly optimized [14,22]. However, the present study shows that analysis of Rubisco optimization necessitates considering the predominant environmental conditions, which influence the \([CO_2]\) and temperature conditions at the site of carboxylation. When including physiologically relevant conditions of chloroplastic \([CO_2]\) and temperature, the analysis reveals that Rubisco is not that perfectly optimized, and that there is room for improvement. These results are in agreement with previous simulation by Zhu et al. [49], and the apparent controversy may depend on whether the analysis compares distant phylogenetic groups [14,22] or is restricted to higher plants [49]. We also demonstrate that current Rubiscos will improve their performance under future climate if change drivers lead to decreased availability of \(CO_2\) for carboxylation. This is likely to occur in future scenarios predicting drier soils, highest increase in temperature and lowest increment in atmospheric \([CO_2]\). Overall, the present results, in line with previous surveys, suggest that the concentration of \(CO_2\) and \(O_2\) in the chloroplast and the leaf temperature have been playing a decisive role in shaping Rubisco evolution.

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### Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at [http://dx.doi.org/10.1016/j.plantsci.2014.01.008](http://dx.doi.org/10.1016/j.plantsci.2014.01.008).

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