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RESEARCH PAPER

Rubisco catalytic properties of wild and domesticated relatives provide scope for improving wheat photosynthesis

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Abstract

Rubisco is a major target for improving crop photosynthesis and yield, yet natural diversity in catalytic properties of this enzyme is poorly understood. Rubisco from 25 genotypes of the Triticeae tribe, including wild relatives of bread wheat (*Triticum aestivum*), were surveyed to identify superior enzymes for improving photosynthesis in this crop. *In vitro* Rubisco carboxylation velocity (V_c), Michaelis–Menten constants for CO_2 (K_c) and O_2 (K_o) and specificity factor ($S_{c/o}$) were measured at 25 and 35 °C. V_c and K_c correlated positively, while V_c and $S_{c/o}$ were inversely related. Rubisco large subunit genes (*rbc*L) were sequenced, and predicted corresponding amino acid differences analysed in relation to the corresponding catalytic properties. The effect of replacing native wheat Rubisco with counterparts from closely related species was analysed by modelling the response of photosynthesis to varying CO_2 concentrations. The model predicted that two Rubisco enzymes would increase photosynthetic performance at 25 °C while only one of these also increased photosynthesis at 35 °C. Thus, under otherwise identical conditions, catalytic variation in the Rubiscos analysed is predicted to improve photosynthetic rates at physiological CO_2 concentrations. Naturally occurring Rubiscos with superior properties amongst the Triticeae tribe can be exploited to improve wheat photosynthesis and crop productivity.

Key words: *Aegilops*, barley, carboxylation, enzyme kinetics, photosynthesis, Rubisco, Triticeae, *Triticum.*

Introduction

Wheat is the most widely grown crop and an important source of protein and calories, providing more than 20% of the calories consumed worldwide (Braun *et al.*, 2010). It is projected that the world population will rise to over 9 billion by the year 2050 (United Nations, Department of Economic and Social Affairs, Population Division, 2013). This growth in population, along with a rise in *per capita* consumption (Kearney, 2010), will increase the global demand for food. Future increases in crop production will rely mainly on new strategies to increase yield and cropping intensity (Gregory and George, 2011; Alexandratos and Bruinsma, 2012; Fischer *et al.*, 2014).

Yield traits that were positively affected by the green revolution appear to have relatively little remaining potential for

further exploitation in modern wheat (Zhu *et al.*, 2010), and further increases in yield potential will need to come from the improvement of photosynthetic efficiency. In this context, significant variation in biomass has been identified in exotic wheat genetic resources (Reynolds *et al.*, 2015). Rubisco, (EC.4.1.1.39) is a key player in photosynthetic CO_2 assimilation, as it catalyses the first step of the Calvin–Benson cycle, fixing carbon dioxide through the carboxylation of ribulose-1,5-bisphosphate (RuBP). Rubisco also catalyses an additional and competing reaction with oxygen, which leads to the loss of fixed carbon and energy during photorespiration. This, together with the relatively low catalytic rate of Rubisco, limits photosynthetic productivity. Overcoming the limitations of Rubisco is therefore a major target in attempts to increase photosynthesis and yield (Parry *et al.*, 2007; Parry *et al.*, 2013).

There is natural variation in the catalytic properties of Rubisco isolated from various higher plants (Delgado *et al.*, 1995; Galmés *et al.*, 2005; Kapralov and Filatov, 2007; Andralojc *et al.*, 2014; Galmés *et al.*, 2014a; Galmés *et al.*, 2014b). Relatively few studies report all catalytic parameters—the maximum velocities (V) and the Michaelis–Menten constants (K_M) for the carboxylase (c) and oxygenase (o) activities (V_c, V_o, K_c, and K_o, respectively) and the specificity factor ($S_{c/o}=(V_c/K_c)/(V_o/K_o)$)—or measurements at anything other than a single temperature. Greater natural diversity is likely to be revealed when the catalytic parameters of Rubisco from a broader range of species become available. Current evidence suggests that there is a trade-off between the maximum carboxylation rate of Rubisco (V_c) and the relative specificity for CO_2 ($S_{c/o}$) (Bainbridge *et al.* 1995; Zhu *et al.*, 2004; Savir *et al.*, 2010), which may limit the extent to which these parameters can be independently altered. Clearly, a superior Rubisco for improving crop performance will have catalytic properties that maximize carboxylation, minimize the oxygenase activity, and enable enhanced rates of photosynthesis in relevant environments (Galmés *et al.*, 2014b; Sharwood and Whitney, 2014).

Evolution of Rubisco variants with differing catalytic properties has been driven by their respective diverse cellular environments, which in turn are affected by their respective external environments (see Carmo-Silva *et al.*, 2014 and references therein). These conditions provide selective pressures which favour changes in Rubisco structure that optimize performance (Tcherkez *et al.*, 2006). While the chloroplast-encoded Rubisco large subunits incorporate the catalytic sites and therefore contribute directly to the observed catalytic properties, recent evidence suggests that changes in expression of genes within the nuclear-encoded small subunit multigene family can also cause catalytic variation (e.g. Morita *et al.*, 2014).

The goal of this study was to identify Rubisco variants in the Triticeae tribe with catalytic properties that are likely to improve photosynthetic efficiency in wheat. We focused on wheat relatives so that useful traits could be introduced into a wheat genetic background by means of wide crossing (thus avoiding genetic manipulation), with increased likelihood that the available (wheat) chloroplast chaperones and Rubisco activase isoforms would subsequently promote the assembly and maintenance of catalytic activity in any resulting forms of the Rubisco holoenzyme. Triticeae genotypes from diverse climates and geographical locations were studied to increase the likelihood of identifying forms of Rubisco with different (and hopefully superior) kinetic properties from those found in *Triticum aestivum* (bread wheat). The resulting catalytic parameters were assessed *in silico* using a biochemical model of leaf photosynthesis. This approach suggested that Rubisco from two of the genotypes studied has the potential to improve the photosynthetic capacity and yield potential of wheat.

Materials and methods

Plant material and growth conditions

For all kinetic measurements, values obtained in test samples were compared with those of *T. aestivum* cv Cadenza, which was used as control. Cadenza is widely grown and has routinely been used in transformation experiments, making it a well-known and characterized variety. A total of 25 genotypes were analysed (Table 1). Species related to bread wheat were chosen with a range of characteristics, such as adaption to warmer conditions (*T. aestivum* SATYN and *T. dicoccon* CIMMYT), or which had been used to introduce desirable traits into bread wheat (Schneider *et al.*, 2008). Seeds were obtained from CIMMYT (Mexico); the Royal Botanic Gardens, Kew (UK); and colleagues at Rothamsted Research. All plants were grown from seed in trays containing Rothamsted Research compost mix in a glasshouse at 20 °C with a 16 h photoperiod. Additional lighting was provided whenever the photosynthetically active radiation (PAR) fell below 500 µmol m^{-2} s^{-1}. All plants were well watered. Young, healthy leaves were harvested 2–3 weeks after sowing and rapidly frozen in liquid nitrogen.

Specificity factor

Rubisco from snap-frozen young leaves (at least 500 cm^2) was extracted in homogenization buffer (40 mM triethanolamine pH 8.0, 10 mM $MgCl_2$, 0.5 mM EDTA, 1 mM KH_2PO_4, 1 mM benzamidine, 5 mM ε-aminocaproic acid, 50 mM 2-mercaptoethanol, 5 mM DTT, 10 mM $NaHCO_3$, 1 mM phenylmethanesulfonyl fluoride (PMSF), 1% w/v insoluble polyvinylpolypyrrolidone (PVPP)) at 0.3 ml cm^{-2}. Leaves were homogenized in a pre-cooled blender for 45 s and then filtered through four layers of muslin. Homogenate was clarified by centrifuging at 13 870×*g* for 12 min at 4 °C, after which PEG_{4000} (60% w/v) was added to the supernatant to obtain a final concentration of 20.5% (w/v). $MgCl_2$ was added to the solution to increase the concentration of $MgCl_2$ to 20 mM. The ensuing protein precipitation was complete after 30 min at 0 °C, and the precipitate collected by centrifugation at 13 870×*g* for 20 min at 4 °C. The protein precipitate was redissolved in column buffer containing 25 mM TEA–HCl (pH 7.8), 5 mM $MgCl_2$, 0.5 mM EDTA, 1 mM ε-aminocaproic acid, 1 mM benzamidine, 12.5% glycerol, 2 mM DTT and 5 mM $NaHCO_3$. This was clarified by centrifugation at 175 000×*g* for 20 min at 4 °C followed by filtration through a 0.45 µm regenerated cellulose syringe filter before further purification by anion-exchange chromatography on a 5 ml HiTrap Q column (GE Healthcare, UK). Rubisco was eluted with a 0–1.0 M linear NaCl gradient in the same buffer. Fractions with significant absorbance at 280 nm were tested for Rubisco activity by measuring the RuBP-dependent incorporation of $^{14}CO_2$ into acid-stable products, as detailed below. Fractions showing Rubisco activity were pooled and further purified by size-exclusion chromatography on a Sephacryl S-200 column (GE Healthcare, UK) using a buffer consisting of 50 mM Bicine–NaOH, pH 8.0, 10 mM $MgCl_2$,

Table 1. *Triticeae genotypes used to survey Rubisco catalytic properties for improving photosynthesis of UK bread wheat (*T. aestivum cv Cadenza*)*

Haploid genome according to Van Slageren (1994). First letter denotes chloroplast genome.

Identity	Species name	Other species name(s) and additional information	Haploid genome
T. aestivum (C)	*Triticum aestivum*	Spring wheat var. Cadenza	BA^uD
T. aestivum SATYN1	*Triticum aestivum* SATYN_II_9410	PUB94.15.1.12/FRTL (CIMMYT line)	BA^uD
T. aestivum SATYN2	*Triticum aestivum* SATYN_II_9440	WHEAR//2*PRL/2*PASTOR (CIMMYT line)	BA^uD
T. aestivum SATYN3	*T. aestivum* SATYN_II_9428	MTRWA92.161/PRINIA/5/SERI*3//RL6010/4*YR/3/PASTOR/4/BAV92 (CIMMYT line)	BA^uD
*T. dicoccon*1	*Triticum dicoccon* CI12214	Emmer wheat; INTRID:CWI47369 ENT:2129 (CIMMYT line)	BA^u
*T. dicoccon*2	*Triticum dicoccon* CI12214	Emmer wheat; INTRID:CWI47368 ENT:2128 (CIMMYT line)	BA^u
*T. dicoccon*3	*Triticum dicoccon* PI355483	Emmer wheat; INTRID:CWI45495 ENT:255 (CIMMYT line)	BA^u
*T. dicoccon*4	*Triticum dicoccon* CI12214	Emmer wheat; INTRID:CWI47366 ENT:2126 (CIMMYT line)	BA^u
T. timonovum	*Triticum timonovum*	Synthetic octoploid of *T. timopheevii*	GA^m
T. timopheevii	*Triticum timopheevii*	Sanduri wheat	GA^m
Triticale (Talentro)	*Secale cereale* × *Triticum aestivum*	× *Triticosecale* cv Talentro; frost and drought tolerant	BA^uR
Triticale (Rotego)	*Secale cereale* × *Triticum aestivum*	× *Triticosecale* cv Rotego; frost and drought tolerant	BA^uR
H. vulgare	*Hordeum vulgare*	Barley var. Lenins; relatively drought tolerant, not cold tolerant	H
Ae. tauschii	*Aegilops tauschii*	*Aegilops squarrosa*; drought tolerant	D
Ae. juvenalis	*Aegilops juvenalis*		DMU
Ae. vavilovii	*Aegilops vavilovii*	Drought tolerant	DMS
Ae. biuncialis	*Aegilops biuncialis*	Drought tolerant	UM
Ae. triuncialis	*Aegilops triuncialis*	Barbed goatgrass; Millenium Seed Bank 47689; winter annual	UC
Ae. comosa	*Aegilops comosa*		M
Ae. uniaristata	*Aegilops uniaristata*		N
S. cereale	*Secale cereale*	Rye var. Agronom; frost and drought tolerant	R
T. monococcum	*Triticum monococcum*	Millenium Seed Bank 11008, einkorn	A^m
Ae. cylindrica	*Aegilops cylindrica*	Jointed goatgrass; cold tolerant	DC
Triticale (Cando)	*Secale cereale* × *Triticum aestivum*	× *Triticosecale* cv Cando; frost and drought tolerant	BA^uR
Ae. speltoides	*Aegilops speltoides*	Not frost tender	S
B. distachyon	*Brachypodium distachyon*	Purple false brome; Accession BD21; diploid inbred	

0.2 mM EDTA, 10 mM $NaHCO_3$ and 2 mM DTT. Peak fractions based on Rubisco activity were pooled and concentrated using Pierce Protein Concentrators (150K MWCO, Thermo Scientific, UK). Samples were snap-frozen in liquid nitrogen and stored at –80 °C. Before use, samples were desalted by gel filtration through Sephadex G50 (medium grade; Sigma-Aldrich, UK) pre-equilibrated with assay buffer (0.1 M Bicine–NaOH, pH 8.2, 20 mM $MgCl_2$).

$S_{c/o}$ was determined by measuring the decline in oxygen that accompanied the total consumption of RuBP in an oxygen electrode (Hansatech Instruments, UK), as described by Parry *et al.* (1989): Rubisco was activated in extracts by adding orthophosphate (4 mM, pH 8.2) and $NaHCO_3$ (11 mM), and incubating at 37 °C for 40 min. A reaction mixture containing assay buffer and carbonic anhydrase (0.001% w/v, ≥2500 W-A units mg protein^{-1}; Sigma-Aldrich, UK) was equilibrated in an oxygen electrode vessel at controlled pH and temperature. All subsequent additions were made through a small aperture using glass syringes. Activated Rubisco and $NaHCO_3$ (2 mM) were added to the vessel and the oxygen signal allowed to stabilize. RuBP (0.37 mM) was added to the reaction, which was allowed to run to completion over a few minutes, as indicated by a stabilized oxygen signal. The amount of RuBP carboxylated was calculated by subtracting the oxygenated amount (represented by the amount of oxygen consumed during the reaction) from the amount added. The specificity factor was calculated as follows:

$$S_{c/o} = (\text{RuBP carboxylated} / \text{RuBP oxygenated}) \times (\text{O}_2\text{concentration} / \text{CO}_2\text{concentration})$$

RuBP was prepared as previously described (Wong *et al.*, 1980).

Rubisco catalytic properties

Rubisco was extracted from 20–30 cm^2 of leaf material that was light-adapted immediately before being snap-frozen, then stored at –80 °C. Leaves were ground in an ice-cold mortar with 100 mg quartz sand and 3.5 ml of ice-cold extraction buffer, consisting of 100 mM Bicine–NaOH, pH 7.9, 5 mM $MgCl_2$, 1 mM EDTA, 2 mM benzamidine, 5 mM ε-aminocaproic acid, 10 mM $NaHCO_3$, 50 mM 2-mercaptoethanol, 5% (w/v) PEG_{4000}, 10 mM DTT, 1% (v/v) plant protease inhibitor cocktail (Sigma-Aldrich, UK), 1 mM PMSF and 2% (w/v) insoluble PVPP. After centrifugation for 5 min at 14 000×*g* and 4 °C, samples were desalted by gel filtration through PD-10 columns (Sephadex G-25 Medium, GE Healthcare, UK) that had been pre-equilibrated with 100 mM Bicine–NaOH, pH 8.0, 10 mM $MgCl_2$, 1 mM EDTA, 1 mM benzamidine, 1 mM ε-aminocaproic acid, 1 mM KH_2P_i, 10 mM $NaHCO_3$, 10 mM DTT and 2% (w/v) PEG_{4000}, 2% (v/v) protease inhibitor cocktail (Sigma-Aldrich, UK) and 20 mM $MgCl_2$ was added before samples were snap-frozen and stored in liquid nitrogen, awaiting assay.

Catalytic parameters were measured essentially as previously described (Carmo-Silva *et al.*, 2010). Carboxylation activity was measured at 8, 16, 24, 36, 68, and 100 µM CO_2 (aq) in equilibrium with a gas phase of N_2 supplemented with 0, 21, 60, or 100% (v/v) O_2. K_M and V_{max} for carboxylation (K_c and V_c, respectively) were calculated at each O_2 concentration using a Michaelis–Menten kinetic model. K_M and V_{max} for oxygenation (K_o and V_o, respectively) were calculated as follows:

$$K_o = [\text{O}_2] / \left[\left(K_{M,app} / K_c \right) - 1 \right]$$

and

$$V_o = \left(V_c \times K_o \right) / \left(K_c \times S_{c/o} \right)$$

where K_c is the Michaelis–Menten constant for CO_2 in the absence of O_2, and $K_{M,app}$ is the apparent Michaelis–Menten constant for CO_2 as measured in the reactions equilibrated with 21, 60, or 100% O_2. Specific mixtures of N_2 and O_2 were prepared using a gas divider (Signal Group, UK) and concentrations of O_2 in solution were calculated at 100% relative humidity and standard atmospheric pressure (101.3 kPa). At 25 °C, the solubility of O_2 was taken as 257.5 µM and the saturation vapour pressure of water as 11.6 kPa. At 35 °C, the solubility of O_2 was taken as 216.6 µM and the saturation vapour pressure of water as 12.0 kPa (http://www.eidusa.com/Theory_DO.htm). The concentration of CO_2 in solution (in equilibrium with HCO_3^-) was calculated assuming a pK_a of 6.11 at 25 °C and a pK_a of 6.06 at 35 °C for carbonic acid, taking into consideration the pH of each buffer solution (measured on the day of assay). Carbonic anhydrase (≥77 WA units per 1 ml reaction; Sigma-Aldrich, UK) was present in the reaction solution to maintain equilibrium between $NaHCO_3$ and CO_2. Control reactions were performed by measuring CO_2 fixation (acid-stable ^{14}C) in reaction solutions lacking RuBP or $NaHCO_3$, as well as following total inhibition of Rubisco by prior treatment with an excess of the tight-binding inhibitor 2-carboxyarabinitol-1,5-bisphosphate (CABP). These controls confirmed that the activity measured was entirely due to Rubisco.

Radioactive content of ^{14}C-labelled compounds was measured in 0.4–0.45 ml aqueous solutions to which were added 3.6 ml Ultima Gold Scintillation cocktail (Perkin-Elmer, UK), in a Tri-Carb 2100TR Liquid Scintillation Analyser (Perkin-Elmer, USA).

Turnover number (k_{cat}: mol product mol active site^{-1} s^{-1}) was calculated from the corresponding V_{max} values (V_c and V_o: µmol acid-stable ^{14}C mg Rubisco^{-1} min^{-1}).

Rubisco quantification

Rubisco was quantified by the [^{14}C]CABP binding assay described by Parry *et al.* (1997). For this, aliquots of the leaf extracts used in the assays described above, which had been snap-frozen immediately after extraction, were used. Each assay was performed in duplicate. Radioactive content of ^{14}C-labelled compounds was measured as described above in 'Rubisco catalytic properties'. Radiolabelled [2′-^{14}C]CABP was prepared as previously described (Pierce *et al.*, 1980).

*Sequencing of Rubisco large subunit genes (*rbc*L)*

Genomic DNA was extracted from young leaf tissue using the Qiagen DNEasy Plant Kit (Qiagen, UK). Partial *rbc*L fragments (equivalent to codons 1–463, *c.* 98% of the *rbc*L coding region) were amplified (Phusion HF polymerase, Invitrogen, USA) using the primers 5′rbcL_F2 (5′-TAATTCATGAGTTGTAGGGAGGG-3′) and cp063R (5′-TTTCCATACTTCACAAGCAGCAGCTAG-3′, from Dong *et al.*, 2013), and cloned using the pGEM T-Vector Easy System (Promega, UK) with blue-white selection. For each genotype, multiple colonies with the fragment incorporated were identified and sequenced using the Eurofins Genomics service (Eurofins Genomics EU, Germany). Sequencing was performed using the primers M13 rev (5′-CAGGAAACAGCTATGACC-3′), M13 uni (5′-TGTAAAACGACGGCCAGT-3′), DRS15 (5′-CAAAAGTAGTAGAAACCATTTTAGTTCAGGTGG-3′ and DRS19 (5′-GKGYTCCTATTGTAATGCATGACTACTTAAC-3′). Sequence data were analysed using Geneious 7 (Biomatters; Kearse *et al.*, 2012). Sequences obtained have been submitted to EMBL (http://www.ebi.ac.uk/ena/) and are publicly available (see Supplementary Table S1 at *JXB* online for accession numbers). Corresponding residue differences in the predicted large subunit (LSu) sequences appear in the format [Cadenza residue][residue position][test species residue] throughout the text.

Photosynthesis modelling

The effect of replacing native Rubisco in a wheat leaf with Rubisco from another species was modelled at 25 and 35 °C by entering the measured Rubisco catalytic constants into the biochemical models of carboxylation-limited and RuBP-limited C_3 photosynthesis (equations 2.20 and 2.23, respectively, in von Caemmerer (2000)). To accomplish this, values of K_c, K_o, and $S_{c/o}$ were converted from units of concentration (mol l^{-1}) to those of partial pressure (bar), assuming solubilities of 3.34×10^{-2} and 1.26×10^{-3} mol (l bar)$^{-1}$ for CO_2 and O_2, respectively, for assays performed at 25 °C. At 35 °C, the respective solubilities were taken as 2.51×10^{-2} and 1.083×10^{-3} mol (l bar)$^{-1}$. We assigned a value of 38 µmol m^{-2} for the estimated number of Rubisco active sites and kept this value constant for all samples. R_d was calculated as $0.015V_{cmax}$. We assumed J_{max} as $1.5V_{cmax}$ at 25 °C and 35 °C giving a good fit above C_a. Equations used to generate the A–C_i curves were:

$$A_c = < \left[(C_i - \Gamma^*)V_{cmax}\right] / \left\{C_i + \left[K_c\left(1 + O_2 / K_o\right)\right]\right\} > - R_d$$

and

$$A_j = \left\{\left[(C_i - \Gamma^*)J_{max}\right] / \left(4C_i + 8\Gamma^*\right)\right\} - R_d$$

(von Caemmerer, 2000).

Statistical methods

Best-fit values of Michaelis–Menten constants (K_c and K_o) and maximum velocities (V_c and V_o) were derived from the kinetic data using Sigmaplot (v12.5). There was one determination per test genotype, with Cadenza values calculated from n=7 for catalytic properties and n=9 for $S_{c/o}$. Values of $S_{c/o}$ at 25 °C were normalized to the corresponding value for the Rubisco of *T. aestivum* cv Cadenza ($S_{c/o}$=100), which was determined in parallel to each test sample measured (Parry *et al.*, 1989). For $S_{c/o}$, the mean±SEM for every Rubisco preparation was calculated from a minimum of five technical replicates. Correlation coefficients were calculated using the Pearson product moment correlation test. The interaction between genotype and temperature was analysed using a non-parametric statistical approach. Ranking was done in descending order with the highest rank assigned number 1. Ranks of the measured variables for each genotype at 25 °C and 35 °C were correlated using Spearman's rank correlation coefficient and these were tested for statistical significance using Genstat (17th edn, VSN International Ltd, Hemel Hempstead, UK).

Results

Rubisco catalytic properties at 25 and 35 °C were determined for 25 genotypes of Triticeae. For all genotypes, the maximum carboxylation velocity (V_c) was significantly higher at 35 °C than at 25 °C, ranging from 1.34 times higher in *Ae. juvenalis* to 2.65 times higher in *T. dicoccon3* (Fig. 1 and Supplementary Tables S2 and S3). At 25 °C, *H. vulgare* ranked the highest for V_c, followed by *Ae. cylindrica*, *T. aestivum* SATYN3, and Triticale (Cando) above *T. aestivum* cv Cadenza (reference genotype). At 35 °C, *T. dicoccon*4 (a line developed by CIMMYT for warm climates) ranked the highest for V_c, followed by the other CIMMYT lines (*T. aestivum* SATYN and *T. dicoccon* genotypes) and *H. vulgare*, before Cadenza, which ranked seventh. At both temperatures *B. distachyon* ranked the lowest for V_c.

There is statistical evidence of a correlation between the performance of the genotypes with respect to V_c across temperature, with a Spearman's rank correlation coefficient ρ=0.707 (P<0.001) (see Supplementary Tables S2 and S3).

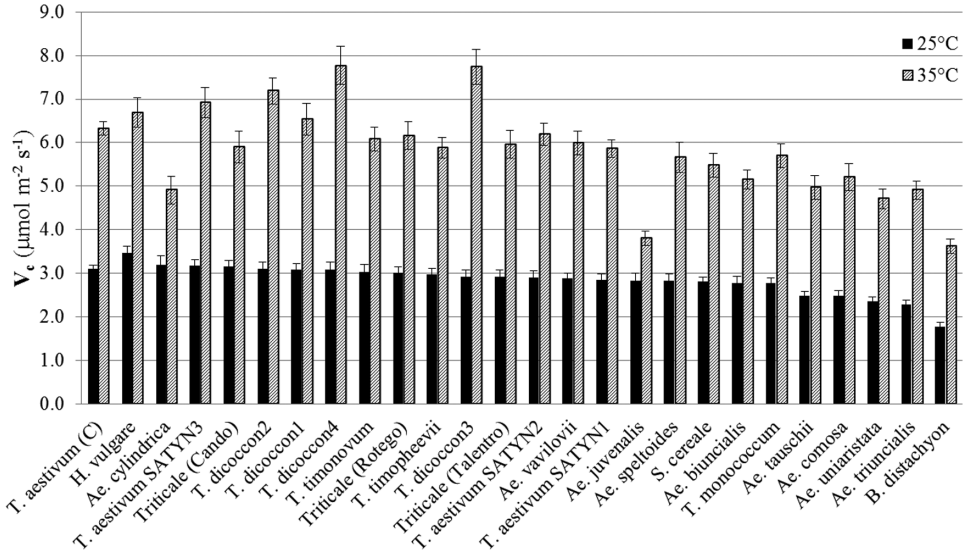

Fig. 1. Rubisco carboxylation velocity (V_c) at 25 °C (black bars) and 35 °C (hatched bars) in 25 Triticeae genotypes. Data organized in decreasing rank at 25 °C, except for *T. aestivum* cv Cadenza, which is shown on the far left-hand side for comparison.

This value was slightly lower when comparing genotypes grouped according to *rbc*L sequence, although still significant (ρ=0.607, *P*=0.035) (Table 2).

Rubisco from 14 genotypes had a higher affinity for CO_2 (lower K_c) than Cadenza at 25 °C, with *B. distachyon* ranking the highest (see Supplementary Table S2). Similarly, at 35 °C 13 genotypes showed a higher affinity for CO_2 (lower K_c) compared with Cadenza (Supplementary Table S3). Of these, only Rubisco from *H. vulgare* showed both a higher V_c and a higher affinity for CO_2 compared with Cadenza based on rank.

Regardless of the measurement temperature, Rubisco from most of the genotypes had a lower maximum oxygenation velocity (V_o) than Cadenza. Rubisco from genotypes that had a low affinity for O_2 (i.e. a high K_o) at 25 °C also showed a relatively low affinity for O_2 at 35 °C (Table 2 and Supplementary Tables S2 and S3).

Rubisco specificity factor ($S_{c/o}$) ranged from 90.4 (for *Ae. juvenalis*) to 111.0 (for *B. distachyon*) at 25 °C and was lower at 35 °C for all species (Fig. 2 and Supplementary Tables S2 and S3), ranging from 68.8 for *T. aestivum* SATYN1 to 94.0 for *B. distachyon*. In contrast to its ranking with respect to V_c, *B. distachyon*, ranked the highest for $S_{c/o}$ at both temperatures. The CIMMYT lines *T. aestivum* SATYN3, *T. dicoccon*1, and *T. dicoccon*2 ranked much higher at 35 °C than at 25 °C with respect to $S_{c/o}$, although only *T. dicoccon*1 and *T. dicoccon*2 ranked higher than Cadenza. A correlation was identified in the performance of the genotypes across temperature with respect to $S_{c/o}$ (ρ=0.857, *P*=0.003; Fig. 2 and Supplementary Tables S2 and S3). The correlation coefficient for $S_{c/o}$ across temperature was lower but still significant when genotypes were grouped according to *rbc*L (ρ=0.503, *P*=0.002; Table 2). To compare $S_{c/o}$ across temperatures, the respective ranks of individual genotypes were added up and compared (see Supplementary Tables S2 and S3). This revealed that *B. distachyon* (sum of ranks=2), *Ae. tauschii* (sum of ranks=8), *T. monococcum* (sum of ranks=8), *Ae. cylindrica* (sum of ranks=9) and *Ae. triuncialis* (sum of ranks=10) maintained their ranking much better than Cadenza (sum of ranks=28) across different temperatures.

A positive correlation was observed between Rubisco V_c and K_c for all 25 genotypes, with this correlation being stronger at 35 °C (*r*=0.798, *P*<0.001) than at 25 °C (*r*=0.372, *P*=0.062) (Fig. 3). Rubisco from two genotypes (*Ae. cylindrica* and *H. vulgare*) appeared to have superior catalytic properties at 25 °C, possessing higher V_c and lower K_c values than Cadenza. From these, only Rubisco from *H. vulgare* retained superior properties at 35 °C compared with Cadenza, which performed remarkably well at this higher temperature. A strong positive correlation was found between V_c and V_o at 25 °C (*r*=0.726, *P*<0.001), while a moderate negative correlation was found between V_c and $S_{c/o}$ at both temperatures (*r*=–0.428, *P*=0.029 at 25 °C and *r*=–0.528, *P*=0.006 at 35 °C).

Analysis of the *rbc*L coding sequences (codons 1–463) of the 25 genotypes revealed differences relative to the Cadenza reference sequence in 12 corresponding large subunit (LSu) residues, at positions spanning a number of domains within the Rubisco large subunit structure (Table 3). For 11 genotypes, all of which were either *Triticum* or Triticale, LSu sequences were identical to Cadenza at the amino acid level (Table 3 and Supplementary Table S1). Of the remaining 14 genotypes, each possessed at least one different amino acid from the Cadenza LSu. The LSu residue differences K14Q and S95N were the most common, and were found to occur together in all but one *Aegilops* species. These two residue differences were also found in *S. cereale* and *T. monococcum*. In *H. vulgare*, K14Q was the only difference relative to Cadenza *rbc*L. In *Ae. cylindrica*, in addition to K14Q and S95N, the LSu sequence also contained the difference V17A compared with Cadenza.

The catalytic efficiency of Rubisco in air (21% O_2) can be measured as the ratio between the carboxylase turnover

Table 2. *Kinetic parameters of Rubisco at 25 and 35 °C according to the respective* rbcL *sequence (residues 1–463)*

Where $n>1$, values are means±SEM for the Triticiae Rubiscos containing the same *rbc*L sequence. Other kinetic parameters are calculated using the Michaelis–Menten kinetic model as explained in text. For $S_{c/o}$, $n\geq5$ technical replicates per genotype.

Assay temp	Representative genotype	*n*	K_c (μM)	V_c (μmol min^{-1} mg^{-1})	K_o (μM)	V_o (μmol min^{-1} mg^{-1})	$S_{c/o}$	k_{cat}/K_c (21% O_2)	Rank (K_c)	Rank (V_c)	Rank ($S_{c/o}$)
25 °C	*T. aestivum* cv Cadenza	12	16.3±0.4	3.01±0.03	431.6±12.3	0.85±0.02	95.94±0.96	0.14±0.00	6	4	7
	H. vulgare	1	15.2±2.4	3.47±0.16	465.3±27.8	1.09±0.14	101.96±5.21	0.17	3	1	4
	Aegilops spp.	9	15.6±1.0	2.63±0.08	431.4±17.6	0.74±0.02	100.84±2.20	0.13±0.01	4	6	5
	Ae. cylindrica	1	13.7±2.9	3.2±0.20	451.0±11.4	0.97±0.02	108.9±3.80	0.17	2	2	2
	Triticale (Cando)	1	16.1±2.4	3.15±0.14	384.0±4.7	0.75±0.01	99.73±5.08	0.14	5	3	6
	Ae. speltoides	1	16.5±3.2	2.82±0.17	446.9±11.0	0.84±0.02	102.3±5.6	0.13	7	5	3
	B. distachyon	1	11.9±2.5	1.78±0.10	395.7±18.8	0.54±0.03	111±4.00	0.1	1	7	1
35 °C	*T. aestivum* cv Cadenza	12	28.5±1.6	6.55±0.20	363.2±7.1	1.07±0.03	79.69±2.38	0.18±0.01	7	2	6
	H. vulgare	1	24.4±3.2	6.69±0.34	315.2±15.6	0.97±0.05	89.4±4.16	0.2	6	1	2
	Aegilops spp.	9	23.0±0.8	5.1±0.21	382.1±10.6	1.01±0.05	87.09±1.23	0.17±0.01	4	5	5
	Ae. cylindrica	1	20.7±3.7	4.91±0.32	310.1±2.8	0.83±0.01	89.1±1.60	0.17	2	6	3
	Triticale (Cando)	1	21.5±3.7	5.9±0.37	360.1±26.0	1.30±0.09	76.14±2.76	0.2	3	3	7
	Ae. speltoides	1	23.9±4.0	5.66±0.35	382.1±21.7	1.02±0.06	88.5±2.2	0.18	5	4	4
	B. distachyon	1	18.1±2.5	3.62±0.17	431.7±27.3	0.92±0.06	94±1.80	0.16	1	7	1

number and the Michaelis–Menten constant for CO_2 (i.e. k_{cat}/K_c at 21% O_2, Table 2). Rubisco from *Ae. cylindrica* and *H. vulgare* appeared to have a superior efficiency to Cadenza (and other genotypes with the reference LSu sequence) at 25 °C. Rubisco from *H. vulgare* also showed superior efficiency at 35 °C, along with Triticale (Cando).

From the relationship between catalytic efficiency (k_{cat}/K_c at 21% O_2) and $S_{c/o}$ it is also possible to identify Rubisco enzymes with superior catalytic performance (Fig. 4). In general, catalytic efficiency was higher, but $S_{c/o}$ was lower, at 35 °C compared with 25 °C. Forms of Rubisco with the same LSu sequence (residues 1–463) showed considerable variation in their combination of catalytic properties. Rubisco from *H. vulgare*, which only differs from Cadenza by virtue of the alternative residue Q14 (K14 in Cadenza), stood out as having a promising combination of k_{cat}/K_c and $S_{c/o}$ at both temperatures. Rubisco from *Ae. cylindrica*, differing by K14Q, V17A and S95N relative to Cadenza, showed promise only at 25 °C. Rubisco from *Ae. vavilovii* showed a similar catalytic response, despite the LSu sequence being identical to that from a number of other species that did not show such catalytic advantage compared with Cadenza. In *B. distachyon*, six residue changes compared with Cadenza (Table 3) might be associated with a higher $S_{c/o}$, but at the expense of catalytic efficiency (Fig. 4).

The Rubisco catalytic constants measured *in vitro* at 25 and 35 °C for Cadenza, *Ae. cylindrica* and *H. vulgare* were used to assess the theoretical impact on photosynthetic performance of wheat leaves over a range of intercellular CO_2 concentrations, including those corresponding to ambient air (*c.* 210 μbar). This modelling exercise predicted that at 25 °C photosynthetic rates would be improved by replacing wheat Rubisco with the enzyme from either *Ae. cylindrica* or *H. vulgare* (Fig. 5A, B). At 25 °C Rubisco from *Ae. cylindrica* showed a maximal increase in assimilation rate of 23% (6.3 μmol m^{-2} s^{-1}) compared with Cadenza at 270 μbar C_i (Fig. 5A), while Rubisco from *H. vulgare* maximally increased assimilation rate by 22% (6.7 μmol m^{-2} s^{-1}) at 300 μbar C_i (Fig. 5B). Rubisco from *H. vulgare* also showed promise for the improvement of photosynthesis in wheat at 35 °C, while the enzyme from *Ae. cylindrica* was inferior to Cadenza wheat at the higher temperature (Fig. 5C, D).

Discussion

The catalytic properties and primary sequence of the Rubisco large subunits (LSu, encoded by *rbc*L) from 25 Triticeae genotypes revealed diversity relevant to improving wheat photosynthetic performance in current and projected warmer temperatures. In the major wheat producing countries, grain filling is accompanied by increasing daytime temperatures (Asseng *et al.*, 2015). Within the limits of resources available to this study, measurements were taken at an ideal growth temperature (25 °C, Nagai and Makino, 2009), and at an elevated temperature at which a pronounced negative impact on yield would be expected (35 °C, Duncan *et al.*, 2014). At the higher temperature V_c was higher, but $S_{c/o}$ was lower, which

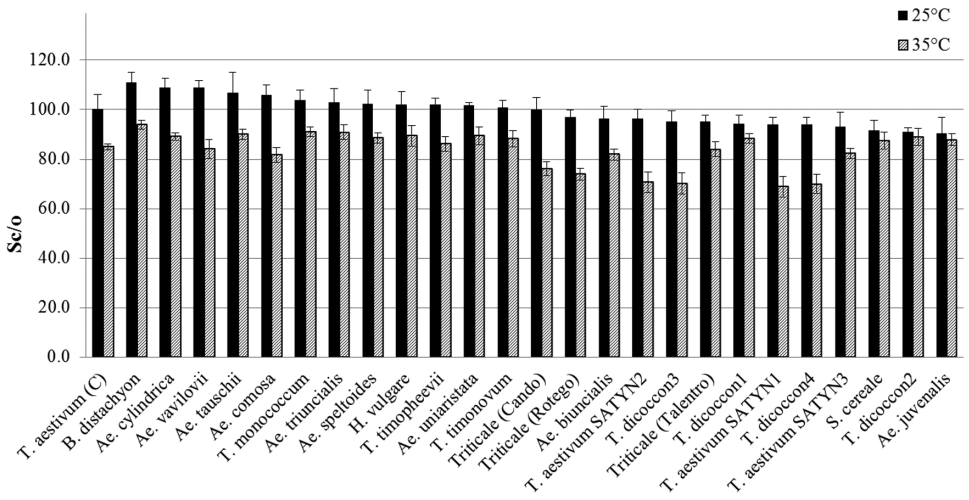

Fig. 2. Specificity factor of Rubisco ($S_{c/o}$) at 25 °C (black bars) and 35 °C (hatched bars) in 25 Triticeae genotypes. Data organized in decreasing rank at 25 °C, except for *T. aestivum* cv Cadenza, which is shown on the far left-hand side for comparison.

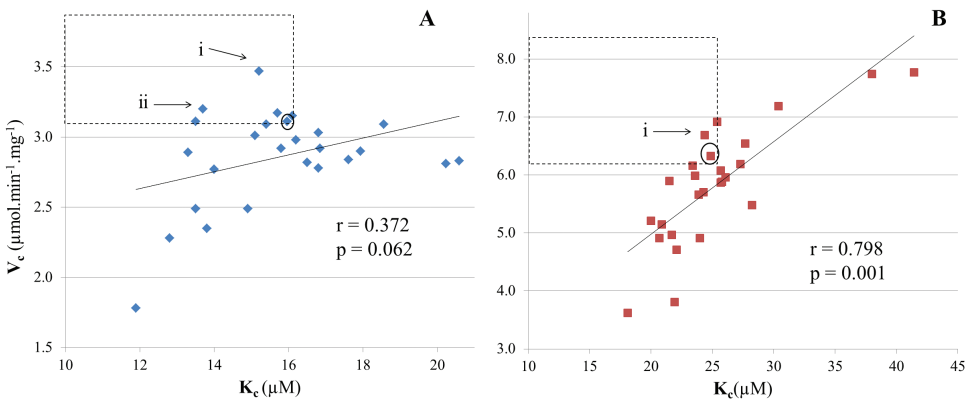

Fig. 3. Relationship between V_c and K_c for Rubisco from 25 Triticeae genotypes at 25 °C (A) and 35 °C (B) in the absence of O_2. Regression lines indicate the best fit through the data. Correlation coefficients (*r*) and *P*-values shown. The data point represented by *Triticum aestivum* cv Cadenza is highlighted by a circle. The area in the graph where Rubiscos with superior characteristics would be found is outlined. Arrows indicate genotypes with potentially superior Rubisco properties compared with Cadenza wheat. (i) *H. vulgare*; (ii) *Ae. cylindrica*.

is consistent with previous research (Brooks and Farquhar, 1985; Tcherkez *et al.*, 2006; Savir *et al.*, 2010; Galmés *et al.*, 2014b). Differences were observed in the Rubisco response to temperature that suggests some acclimation to different geographical locations.

As reported previously (Savir *et al.*, 2010), a positive correlation between V_c and K_c, the determinants of Rubisco carboxylase efficiency, was observed at both temperatures for the Triticeae genotypes studied here, indicating that genotypes with a high V_c tend to have lower affinity for CO_2. The catalytic efficiency of Rubisco in air was a useful tool to identify Rubiscos with superior performance. Furthermore, the combined results suggest that all of the Rubisco catalytic properties, including the specificity factor, must be taken into account during the search for forms of Rubisco with improved performance in air. This follows from the parameters required for biochemical modelling of photosynthetic performance, which include V_c, K_c, and $S_{c/o}$ ($=V_c.K_o/V_o.K_c$), the latter being used to determine the compensation point ($\Gamma^*=0.5[O_2]/S_{c/o}$) in the absence of dark respiration (von Caemmerer, 2000).

In wheat, variation in V_c has been observed across different genotypes and it has been suggested that many of the catalytic properties of Rubisco are determined by the large subunit (Evans and Austin, 1986; Terachi *et al.*, 1987; Kasai *et al.*, 1997), which contains the catalytic sites. The *rbc*L gene is chloroplast encoded (Spreitzer and Salvucci, 2002) and the chloroplast genome tends to be evolutionarily highly conserved. However, within the Poaceae, *rbc*L has evolved at a relatively rapid rate compared with other families of flowering plants (Bousquet *et al.*, 1992; Gaut *et al.*, 1992). Since the large subunits contribute directly to catalytic function, variation in this sequence in wheat relatives represents a potential source of improved catalytic activity.

The majority of the Triticeae genotypes characterized in this study are highly inter-related and this was reflected in the similarity of the respective *rbc*L sequences. Some of the observed differences in Rubisco catalytic activity correlated with differences in *rbc*L sequence. For example, differences in catalytic properties determined for *H. vulgare*, *Ae. cylindrica*, Triticale (Cando) and *B. distachyon* compared with Cadenza Rubisco might be associated with their specific *rbc*L sequences. Conversely, differences in catalysis for genotypes with the same *rbc*L sequence (e.g. the Cadenza group represented by black symbols or the *Aegilops* group represented by yellow

Table 3. *Amino acid differences in the Rubisco large subunit predicted protein sequences for 25 Triticeae genotypes relative to* T. aestivum *cv Cadenza*

Residues under positive selection (Kapralov and Filatov, 2007, Galmés *et al.* 2014*b*) are indicated with an asterisk. Functional interactions described in the literature for these residues as indicated (AS, active site; ID, intradimer interactions; DD, dimer:dimer interactions; RA, interactions with Rubisco activase; SSU, interaction with small subunits). Symbols and colours match those used in Fig. 4. na, not applicable.

Residue change	Symbol	Interaction	Location of residue	Species
na	■◆			*T. aestivum* cv. Cadenza
na	▲●			*T. aestivum* SATYN1
				T. aestivum SATYN2
				T. aestivum SATYN3
				*T. dicoccon*1
				*T. dicoccon*2
				*T. dicoccon*3
				*T. dicoccon*4
				T. timonovum
				T. timopheevii
				Triticale (Talentro)
				Triticale (Rotego)
K14Q*	▲●		N-terminal	*H. vulgare* cv. Lenins
K14Q*	▲●		N-terminal	*Ae. tauschii*
S95N*		ID, RA		*Ae. juvenalis*
				Ae. vavilovii
				Ae. biuncialis
				Ae. triuncialis
				Ae. comosa
				Ae. uniaristata
				S. cereale cv. Agronom
				T. monococcum
K14Q*	▲●		N-terminal	*Ae. cylindrica*
V17A			N-terminal	
S95N*				
G47W	▲●	ID	Strand B	*Triticale* (Cando)
K81R	▲●			*Ae. speltoides*
I225T*		SSU	Helix 2	
G10S	▲●		N-terminal	*B. distachyon*
K21R			N-terminal	
A91P*		RA		
I251M*		DD, ID, SSU	Helix 3	
S328A*		AS	Loop 6	
M341I		AS, ID	Helix 6	

symbols in Fig. 4) may be associated with either changes in the extreme C-terminus whose sequence was not determined or, more likely, diversity in the small subunit sequence (Guo *et al.*, 1997; Spreitzer, 2003; Spreitzer *et al.*, 2005; Ishikawa *et al.*, 2011; Cai *et al.*, 2014; Morita *et al.*, 2014). Future studies to characterize the exact number and relative expression of small subunit genes in wheat and wheat relatives may reveal novel avenues for improving Rubisco catalysis and photosynthesis.

When comparing kinetic parameters between species grouped by LSu sequence (Table 2), *H. vulgare* (difference K14Q) ranked highest in V_c at both 25 and 35 °C, with only *Ae. cylindrica* (K14Q, V17A, and S95N) also ranking higher than species with the control sequence at 25 °C. Rubisco from *Ae. cylindrica* also had a lower K_c (higher affinity for CO_2) at both temperatures compared with Rubisco from species with the reference *rbc*L sequence.

When present as lysine, large subunit residue 14 is known to be a site of post-translational tri-methylation in many flowering plant species, but not *T. aestivum* (Houtz *et al.*, 2008). Available data show that glutamine is the only alternative residue found at this position (K14Q, Houtz *et al.*, 1989; Houtz *et al.*, 1992; Trievel *et al.*, 2003), although the importance of this position and its modification remains unresolved (Houtz *et al.*, 2008). The results presented here suggest the possibility that either the amino acid difference itself (K14Q) or the absence of methylation at this position alters Rubisco kinetics in a manner favourable to photosynthesis.

The other residue difference common to most of the *Aegilops*, *S. cereale* and *T. monococum*, S95N, occurs in a poorly conserved region of the *rbc*L gene, which is in the proximity of residues known to be involved in interactions with Rubisco activase (Portis, 2003; Portis *et al.*,

2008; Carmo-Silva *et al.*, 2014). Recently, this residue was highlighted during a search for residues under positive selection, and it is in proximity to residues involved in L_2 intradimer interactions (Galmés *et al.*, 2014*b*).

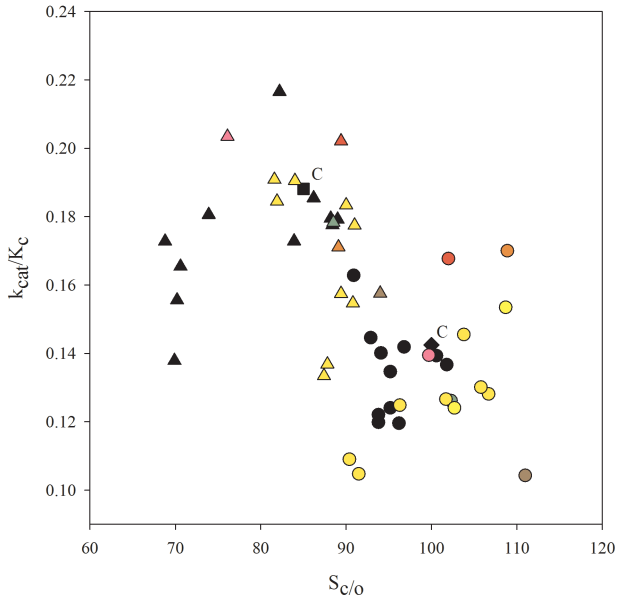

Fig. 4. The relationship between the catalytic efficiency of Rubisco at 21% O_2 (k_{cat}/K_c, μM s^{-1}) and the specificity factor ($S_{c/o}$) of Rubisco at 25 °C (circles) and 35 °C (triangles). Each colour denotes an *rbc*L sequence (as per Table 3) and Cadenza wheat (C, used as reference) is represented by the diamond and square at 25 and 35 °C, respectively.

Interestingly, species combining the K14Q and S95N residue differences (but having no other differences) showed no consistent catalytic difference compared with the reference LSu sequence group.

The valine at position 17 is known to be involved in intradimer interactions (Knight *et al.*, 1990; Kellogg and Juliano, 1997). Rubisco from *Ae. cylindrica* containing V17A in combination with K14Q and S95N had improved carboxylation catalytic efficiency at 21% O_2 and 25 °C compared with the reference Cadenza (Table 2). This form of Rubisco was predicted by modelling to improve photosynthetic performance at all CO_2 levels relative to Cadenza at 25 °C (Fig. 5A). Hence, the influence exerted by the relatively conservative Val–Ala (V17A) difference, when combined with K14Q (and S95N), may explain the positive impact on Rubisco catalysis.

H. vulgare Rubisco had improved k_{cat}/K_c compared with Cadenza, while only containing the K14Q difference. Studies in *Anacystis nidulans* found that a K14Q or K14L mutation had no influence on enzyme activity (Kettleborough *et al.*, 1991). While the present study did not cover the extreme C-terminus of the large subunit, available sequences showed a KV extension in that region of the *H. vulgare* sequence (Petersen and Seberg, 2003), which may be relevant to its superior catalysis in comparison to Cadenza. Confirmation of this hypothesis would be valuable, given that modelling of the photosynthetic response to intercellular CO_2 predicts a benefit at both 25 and 35 °C by replacing the native Rubisco with the barley enzyme.

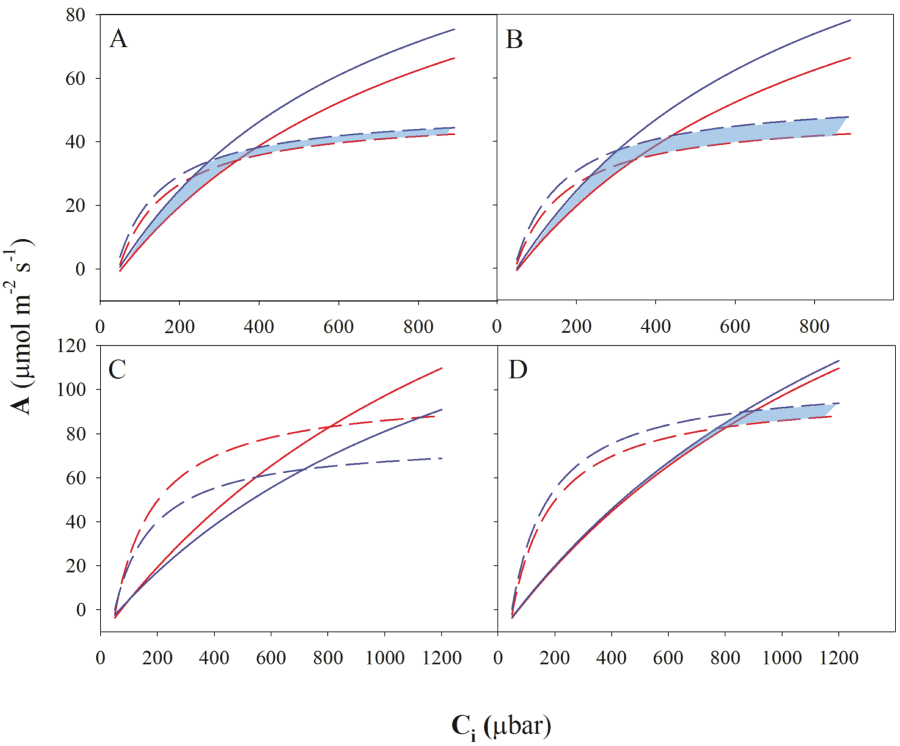

Fig. 5. Modelling photosynthesis at 25 °C (A, B) and 35 °C (C, D), to demonstrate the benefit of replacing Rubisco of *T. aestivum* cv Cadenza (red) with Rubisco from *Ae. cylindrica* (A, C; blue) or *H. vulgare* (B, D; blue). Rubisco-limited (A_c, solid lines) and RuBP regeneration-limited (A_j, dashed lines) rates of net CO_2 assimilation (A) were derived using the model of Farquhar et al. (1980) and the Rubisco catalytic constants measured *in vitro* for each genotype. Blue shading indicates where Rubisco from the test genotypes showed higher assimilation rates than native Cadenza Rubisco.

As with *Ae. cylindrica* (Colmer *et al.*, 2006), barley is considered to be a valuable genetic resource for improving stress tolerance in wheat (Dulai *et al.*, 2011; Molnar-Lang *et al.*, 2014). Barley–wheat hybrids have been investigated before (Kruse, 1973; Malik *et al.*, 2011; Dulai *et al.*, 2011; Rodríguez-Suárez *et al.*, 2011; Pershina *et al.*, 2012; Zou *et al.*, 2012), but without a focus on yield improvement. One notable exception is the development of Tritordeum, which is a hybrid between wild barley (*H. chilense*) and durum wheat (*T. turgidum ssp. Durum*, haploid genome BA; Martin *et al.* 1996), which has been commercialized (http://www.agrasys.es/). Tritordeum has particularly high protein content and has shown tolerance to drought conditions in field trials (Martin *et al.*, 1999; Villegas *et al.*, 2010). While Tritordeum does not include the D genome present in *T. aestivum*, data in this study suggest that this hybrid warrants further investigation with respect to Rubisco kinetics and yield potential.

This study has identified residues that warrant further study, e.g. by mutagenesis. Well targeted single amino acid changes can have a dramatic impact on catalytic performance (e.g. Whitney *et al.*, 2011). However, at present there is no available expression system to test the effect of amino acid substitutions on Rubisco from monocots. An alternative, and possibly more promising approach, which utilizes available technology, is the introgression of traits through wide-crossing of Triticeae genotypes.

Conclusion

The Rubisco catalytic properties determined for 25 genotypes showed that variation exists even amongst closely related genotypes. Rubisco from *Ae. cylindrica* and *H. vulgare* showed promising catalytic properties that should be explored in the context of improving photosynthesis, and ultimately yield, in wheat. Ideally, this could be carried out by crossing a number of the species examined here with bread wheat and studying the resulting plants with respect to Rubisco catalytic activity, photosynthesis and yield. This study supports the case for investment in genetic resource screening for photosynthesis-related characteristics.

Supplementary data

Supplementary data are available at *JXB* online.

Table S1. Rubisco large subunit (*rbc*L) single nucleotide polymorphisms.

Table S2. Rubisco catalytic parameters at 25 °C.

Table S3. Rubisco catalytic parameters at 35 °C.

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References

Alexandratos N, Bruinsma J. 2012. World agriculture towards 2030/2050: the 2012 revision . ESA Working paper No. 12-03. Rome: FAO.

Andralojc PJ, Bencze S, Madgwick PJ, Philippe H, Powers SJ, Shield I, Karp A, Parry MAJ. 2014. Photosynthesis and growth in diverse willow genotypes. Food and Energy Security **3,** 69–85.

Asseng S, Ewert F, Martre P, *et al.* 2015. Rising temperatures reduce global wheat production. Nature Climate Change **5,** 143–147.

Bainbridge G, Madgwick P, Parmar S, Mitchell R, Paul M, Pitts J, Keys AJ, Parry MAJ. 1995. Engineering Rubisco to change its catalytic properties. Journal of Experimental Botany **46,** 1269–1276.

Bousquet J, Strauss SH, Doerksen AH, Price RA. 1992. Extensive variation in evolutionary rate of *rbcL* gene sequences among seed plants. Proceedings of the National Academy of Sciences of the United States of America **89,** 7844–7848.

Braun HJ, Atlin G, Payne T. 2010. Multi-location testing as a tool to identify plant response to global climate change. In: Reynolds MP, ed. Climate change and crop production . Wallingford, UK: CABI, 115–138.

Brooks A, Farquhar GD. 1985. Effect of temperature on the CO_2/O_2 specificity of ribulose-1,5-bisphosphate carboxylase/oxygenase and the rate of respiration in the light. Planta **165,** 397–406.

Cai Z, Liu G, Zhang J, Li Y. 2014. Development of an activity-directed selection system enabled significant improvement of the carboxylation efficiency of Rubisco. Protein & Cell **5,** 552–562.

Carmo-Silva AE, Keys AJ, Andralojc PJ, Powers SJ, Arrabaca MC, Parry MAJ. 2010. Rubisco activities, properties, and regulation in three different C_4 grasses under drought. Journal of Experimental Botany **61,** 2355–2366.

Carmo-Silva AE, Scales JC, Madgwick P, Parry MAJ. 2014. Optimising Rubisco and its regulation for greater resource use efficiency. Plant, Cell & Environment **38,** 1817–1832.

Colmer TD, Flowers TJ, Munns R. 2006. Use of wild relatives to improve salt tolerance in wheat. Journal of Experimental Botany **57,** 1059–1078.

Delgado E, Medrano H, Keys AJ, Parry MAJ. 1995. Species variation in Rubisco specificity factor. Journal of Experimental Botany **46,** 1775–1777.

Dong W, Xu C, Cheng T, Lin K, Zhou S. 2013. Sequencing angiosperm plastid genomes made easy: A complete set of universal primers and a case study on the phylogeny of Saxifragales. Genome Biology and Evolution **5,** 989–997.

Dulai S, Molnár I, Molnár-Láng M. 2011. Changes of photosynthetic parameters in wheat/barley introgression lines during salt stress. Acta biologica Szegediensis **55,** 73–75.

Duncan JMA, Dash J, Atkinson PM. 2014. Elucidating the impact of temperature variability and extremes on cereal croplands through remote sensing. Global Change Biology **21,** 1541–1551.

Evans JR, Austin RB. 1986. The specific activity of ribulose-1,5-bisphosphate carboxylase in relation to genotype in wheat. Planta **167,** 344–350.

Farquhar GD, von Caemmerer S, Berry JA. 1980. A biochemical model of photosynthetic CO2 assimilation in leaves of C3 species. Planta **149,** 78–90.

Fischer RA, Byerlee D, Edmeades GL. 2014. Crop yields and global food security: will yield increase continue to feed the world? Canberra: Australian Centre for International Agricultural Research.

Galmés J, Andralojc PJ, Kapralov MV, Flexas J, Keys AJ, Molins A, Parry MAJ, Conesa MA. 2014*a*. Environmentally driven evolution of Rubisco and improved photosynthesis within the C_3 genus *Limonium*. New Phytologist **203,** 989–999.

Galmés J, Flexas J, Keys AJ, Cifre J, Mitchell RAC, Madgwick PJ, Haslam RP, Medrano H, Parry MAJ. 2005. Rubisco specificity factor tends to be larger in plant species from drier habitats and with persistent leaves. Plant, Cell & Environment **28,** 571–579.

Galmés J, Kapralov MV, Andralojc PJ, Conesa MÀ, Keys AJ, Parry MAJ, Flexas J. 2014*b*. Expanding knowledge of the Rubisco kinetics variability in plant species: environmental and evolutionary trends. Plant, Cell & Environment **37,** 1989–2001.

Gaut B, Muse S, Clark WD, Clegg M. 1992. Relative rates of nucleotide substitution at the *rbc*L locus of monocotyledonous plants. Journal of Molecular Evolution **35,** 292–303.

Gregory PS, George TS. 2011. Feeding nine billion: the challenge to sustainable crop production. Journal of Experimental Botany **62,** 5233–5239.

Guo S, Wu G, Wu X. 1997. Rubisco activities and the small subunit gene cloning and functional analysis of *Aegilops squarrosa*. Journal of Integrative Plant Biology **39,** 222–230.

Houtz RL, Magnani R, Nayak NR, Dirk LMA. 2008. Co- and post-translational modifications in Rubisco: unanswered questions. Journal of Experimental Botany **59,** 1635–1645.

Houtz RL, Poneleit L, Jones SB, Royer M, Stults JT. 1992. Posttranslational modifications in the amino-terminal region of the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase from several plant species. Plant Physiology **98,** 1170–1174.

Houtz RL, Stults JT, Mulligan RM, Tolbert NE. 1989. Post-translational modifications in the large subunit of ribulose bisphosphate carboxylase/oxygenase. Proceedings of the National Academy of Sciences of the United States of America **86,** 1855–1859.

Ishikawa C, Hatanaka T, Misoo S, Miyake C, Fukayama H. 2011. Functional incorporation of sorghum small subunit increases the catalytic turnover rate of Rubisco in transgenic rice. Plant Physiology **156,** 1603–1611.

Kapralov MV, Filatov DA. 2007. Widespread positive selection in the photosynthetic Rubisco enzyme. BMC Evolutionary Biology **7,** 73.

Kasai K, Nakamura C, Mori N, Uchida N. 1997. Rubisco activity vs photosynthetic CO_2 assimilation rate in the alloplasmic hybrids of common wheat cv. Chinese Spring. Wheat Information Service **85,** 25–30.

Kearney J. 2010. Food consumption trends and drivers. Philosophical Transactions of the Royal Society. Series B, Biological Sciences **365,** 2793–2807.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Mentjies P, Drummond, A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics **28,** 1647–1649.

Kellogg EA, Juliano ND. 1997. The structure and function of RuBisCO and their implications for systematic studies. American Journal of Botany **84,** 413–428.

Kettleborough CA, Phillips AL, Keys AJ, Parry MAJ. 1991. A point mutation in the N-terminus of ribulose-1,5-bisphosphate carboxylase affects ribulose-1,5-bisphosphate binding. Planta **184,** 35–39.

Knight S, Andersson I, Brändén C-I. 1990. Crystallographic analysis of ribulose 1,5-bisphosphate carboxylase from spinach at 2.4 Å resolution. Subunit interactions and active site. Journal of Molecular Biology **215,** 113–160.

Kruse A. 1973. *Hordeum* × *Triticum* hybrids. Hereditas . **73,** 157–161.

Malik AI, Islam AKMR, Colmer TD. 2011. Transfer of the barrier to radial oxygen loss in roots of *Hordeum marinum* to wheat (*Triticum aestivum*): evaluation of four *H. marinum*–wheat amphiploids. New Phytologist **190,** 499–508.

Martin A, Alvarez JB, Martin LM, Barro F, Ballesteros J. 1999. The development of tritordeum: a novel cereal for food processing. Journal of Cereal Science **30,** 85–95.

Martin A, Martínez-Araque C, Rubiales D, Ballesteros J. 1996. Tritordeum: triticale's new brother cereal. In: Guedes-Pinto H, Darvey N, Carnid VP, eds. Triticale: today and tomorrow . Dordrecht: Kluwer Academic Publishers, 57–72.

Molnar-Lang M, Linc G, Szakacs E. 2014. Wheat-barley hybridization: the last 40 years. Euphytica **195,** 315–329.

Morita K, Hatanaka T, Misoo S, Fukayama H. 2014. Unusual small subunit that is not expressed in photosynthetic cells alters the catalytic properties of Rubisco in rice. Plant Physiology **164,** 69–79.

Nagai T, Makino A. 2009. Differences between rice and wheat in temperature responses of photosynthesis and plant growth. Plant & Cell Physiology **50,** 744–755.

Parry MAJ, Andralojc PJ, Parmar S, Keys AJ, Habash D, Paul MJ, Alred R, Quick WP, Servaites JC. 1997. Regulation of Rubisco by inhibitors in the light. Plant, Cell & Environment **20,** 528–534.

Parry MAJ, Andralojc PJ, Scales JC, Salvucci ME, Carmo-Silva AE, Alonso H, Whitney SM. 2013. Rubisco activity and regulation as targets for crop improvement. Journal of Experimental Botany **64,** 717–730.

Parry MAJ, Keys AJ, Gutteridge S. 1989. Variation in the specificity factor of C_3 higher plant Rubiscos determined by the total consumption of Ribulose-P2. Journal of Experimental Botany **40,** 317–320.

Parry MAJ, Madgwick PJ, Carvahlo JFC, Andralojc PJ. 2007. Prospects for increasing photosynthesis by overcoming the limitations of Rubisco. Journal of Agricultural Science **145,** 31–43.

Pershina LA, Devyatkina EP, Trubacheeva NV, Kravtsova LA, Dobrovolskaya OB. 2012. Characterization of fertility restoration in alloplasmic lines derived from hybridization of self-fertilized offspring of barley-wheat (*Hordeum vulgare* L. × *Triticum aestivum* L.) amphiploid with common wheat varieties Saratovskaya 29 and Pyrotrix 28. Russian Journal of Genetics **48,** 1184–1190.

Petersen G, Seberg O. 2003. Phylogenetic analyses of the diploid species of *Hordeum* (Poaceae) and a revised classification of the genus. Systematic Botany **28,** 293–306.

Pierce J, Tolbert NE, Barker R. 1980. Interaction of ribulose bisphosphate carboxylase/oxygenase with transition state analogues. Biochemistry **19,** 934–962.

Portis AR. 2003. Rubisco activase - Rubisco's catalytic chaperone. Photosynthesis Research **75,** 11–27.

Portis AR, Li C, Wang D, Salvucci ME. 2008. Regulation of Rubisco activase and its interaction with Rubisco. Journal of Experimental Botany **59,** 1597–1604.

Reynolds MP, Tattaris M, Cossani CM, Ellis M, Yamaguchi-Shinozaki K, Saint Pierre C. 2015. Exploring genetic resources to increase adaptation of wheat to climate change. In Ogihara Y, Takumi S, Handa H, eds. Advances in wheat genetics: from genome to field . Springer, 355–368.

Rodríguez-Suárez C, Giménez MJ, Ramírez MC, Martín AC, Gutierrez N, Ávila CM, Martín A, Atienza SG. 2011. Exploitation of nuclear and cytoplasm variability in *Hordeum chilense* for wheat breeding. Plant Genetic Resources **9,** 313–316.

Savir Y, Noor E, Milo R, Tlusty T. 2010. Cross-species analysis traces adaptation of Rubisco toward optimality in a low-dimensional landscape. Proceedings of the National Academy of Sciences of the United States of America **107,** 3475–3480.

Schneider A, Molnár I, Molnár-Láng M. 2008. Utilisation of Aegilops (goatgrass) species to widen the genetic diversity of cultivated wheat. Euphytica **163,** 1–19.

Sharwood RE, Whitney SM. 2014. Correlating Rubisco catalytic and sequence diversity within C_3 plants with changes in atmospheric CO_2 concentrations. Plant, Cell & Environment **37,** 1981–1984.

Spreitzer RJ. 2003. Role of the small subunit in ribulose-1,5-bisphosphate carboxylase/oxygenase. Archives of Biochemistry and Biophysics **414,** 141–149.

Spreitzer RJ, Peddi SR, Satagopan S. 2005. Phylogenetic engineering at an interface between large and small subunits imparts land-plant kinetic properties to algal Rubisco. Proceedings of the National Academy of Sciences of the United States of America **102,** 17225–17230.

Spreitzer RJ, Salvucci ME. 2002. Rubisco: Structure, regulatory interactions, and possibilities for a better enzyme. Annual Review of Plant Biology **53,** 449–475.

Tcherkez GG, Farquhar GD, Andrews TJ. 2006. Despite slow catalysis and confused substrate specificity, all ribulose bisphosphate carboxylases may be nearly perfectly optimized. Proceedings of the National Academy of Sciences of the United States of America **103,** 7246–7251.

Terachi T, Ogihara Y, Tsunewaki K. 1987. The molecular basis of genetic diversity among cytoplasms of *Triticum* and *Aegilops*. VI. Complete nucleotide sequences of the rbcL genes encoding H- and L-type Rubisco large subunits in common wheat and *Ae. crassa* 4x. The Japanese Journal of Genetics **62,** 375–387.

Trievel RC, Flynn EM, Houtz RL, Hurley JH. 2003. Mechanism of multiple lysine methylation by the SET domain enzyme Rubisco LSMT. Nature Structural and Molecular Biology **10,** 545–552.

United Nations, Department of Economic and Social Affairs, Population Division. 2013. World Population Prospects: The 2012 Revision . Volume **I**: Comprehensive Tables ST/ESA/SER.A/336. New York: United Nations.

Van Slageren MW. 1994. Wild wheats: a monograph of *Aegilops L*. and *Amblyopyrum* (Jaub. & Spach) Eig (Poaceae). In Wageningen Agriculture University Papers 94-7 . Wageningen, The Netherlands: Wageningen Agricultural University.

Villegas D, Casadesus J, Atienza SG, Martos V, Maalouf F, Karam F, Aranjuelo I, Nogues S. 2010. Tritordeum, wheat and triticale yield components under multi-local mediterranean drought conditions. Field Crops Research , **116,** 68–74.

Von Caemmerer S. 2000. Biochemical models of leaf photosynthesis . Clayton: CSIRO Publishing.

Whitney SM, Sharwood RE, Orr D, White SJ, Alonso H, Galmes J. 2011. Isoleucine 309 acts as a C_4 catalytic switch that increases ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco) carboxylation rate in Flaveria. Proceedings of the National Academy of Sciences of the Unites States of America **108,** 14688–14693.

Wong CH, McCurry SD, Whitesides GM. 1980. Practical enzymatic synthesis of ribulose-1, 5-bisphosphate and ribose 5 phosphate. Journal of the American Chemical Society **102,** 7939–7940.

Zhu XG, Long SP, Ort DR. 2010. Improving photosynthetic efficiency for greater yield. Annual Review of Plant Biology **61,** 235–261.

Zhu XG, Portis AR, Long SP. 2004. Would transformation of C3 crop plants with foreign Rubisco increase productivity? A computational analysis extrapolating from kinetic properties to canopy photosynthesis. Plant, Cell & Environment **27,** 155–165.

Zou H, Wu Y, Liu H, Lin Z, Ye X, Chen X, Yuan Y. 2012. Development and identification of wheat–barley 2H chromosome translocation lines carrying the Isa gene. Plant Breeding **131,** 69–74.