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Differences between Wheat Genotypes in Specific Activity of Ribulose-1,5-bisphosphate Carboxylase and the Relationship to Photosynthesis

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ABSTRACT

The *in vitro* ribulose-1,5-bisphosphate (RuBP) carboxylase activity per unit of leaf nitrogen was found to be 30% greater in *Triticum aestivum* than in *T. monococcum*. This was due to a higher specific activity of the enzyme from *T. aestivum*, as the amount of RuBP carboxylase protein per unit of total leaf nitrogen did not differ between the genotypes. The occurrence of higher specific activity of RuBP carboxylase is shown to correlate with possession of the large subunit derived from the B genome of wheat.

Despite the greater RuBP carboxylase activity per unit of leaf nitrogen in *T. aestivum*, the initial slopes of curves relating rate of CO_2 assimilation to intercellular $p(CO_2)$ are similar in *T. aestivum* and *T. monococcum* for the same nitrogen content per unit leaf area. The similarity of the initial slopes is the result of a greater resistance to CO_2 transfer between the intercellular spaces and the site of carboxylation in *T. aestivum* than in *T. monococcum*.

Studies of the photosynthetic performance of plants have often been made using a wide variety of species representing diverse evolutionary backgrounds. One advantage of studying the physiology of wheat, apart from the fact that much is already known, is that comparisons can be made between species from different stages in the evolution of the crop. The derivation of modern hexaploid wheat has been well established from a combination of classical genetic and taxonomic work (25) with more recent DNA and protein techniques (5, 30).

Several studies have used a broad series of wheats to examine the changes in photosynthetic capacity that have occurred with domestication and the increase in ploidy. Evans and Dunstone (11) showed that there has been a parallel increase in the size of both seeds and leaves which is associated with a decline in the maximum rate of photosynthesis per unit of leaf area. This decline was thought to be associated with increased mesophyll resistance due to the lower surface to volume ratio of the larger cells in the hexaploid. However, Dunstone *et al.* (8) found the mesophyll resistances to be similar, possibly because greater lobing of the hexaploid cells maintained their surface to volume ratio. Khan and Tsunoda (17) also observed that wild diploid genotyes had higher maximum photosynthetic rates per unit area than modern hexaploids and associated this with greater nitrogen contents per unit of leaf area. More recently, Austin *et al.* (1) have confirmed that the lower photosynthetic rate of the modern wheats is associated with larger leaves and conclude that internal factors, both biochemical and anatomical, contribute to the differences in photosynthetic rate.

This study examines differences in biochemical and anatomical limitations to photosynthesis between primitive and modern lines of wheat. We have concentrated on *in vivo* and *in vitro* analysis of the performance of RuBP[3] carboxylase in relation to photosynthetic capacity of the intact leaf. Differences in photosynthesis between wheat genotypes could result from differential nitrogen allocation to RuBP carboxylase protein, alterations in the kinetic properties of the enzyme, or anatomical differences which affect the CO_2 pressure at the site of carboxylation. We have examined these possibilities through a comparison of a modern hexaploid wheat, *T. aestivum*, and a diploid ancestor, *T. monococcum*. We have also extended this study to include a more thorough examination of the relationship between kinetic properties of RuBP carboxylase and wheat genome.

MATERIALS AND METHODS

Plant Material. Seed of the wheat accessions (Table I) were kindly supplied by Drs. R. A. Fischer, L. T. Evans, and the Australian Wheat Collection, Tamworth. Plants were grown in a glasshouse in spaced 5-L pots of sterilized soil. For each accession, there were four or more replicate pots, each with two plants per pot. The plants were watered daily and given 500 ml of normal nutrient solution three times a week. Penultimate or flag leaves of *T. aestivum* cv Yecora 70 and *T. monococcum* cv Einkorn 292 were used for gas exchange measurements. RuBP carboxylase was extracted from these leaves on the following morning. In a subsequent experiment, specific activities of RuBP carboxylase were determined in extracts of the youngest fully expanded leaves collected from 8-week-old plants, with the exception of *T. urartu* plants which were 20 weeks old. With *T. urartu* and *T. aestivum*, these were penultimate or flag leaves.

Gas Exchange. CO_2 and water vapor exchange were measured in an open gas exchange system with two double-sided leaf chambers (for details, see 9). Differences in the $p(CO_2)$ and $p(H_2O)$ entering and leaving the leaf chambers were measured with IR gas analyzers (Beckman 865 for CO_2 and ADC series 225 for H_2O). The $p(CO_2)$ of the air entering the leaf chambers

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[3] Abbreviations: RuBP, ribulose1,5-bisphosphate; CABP, carboxyarabinatol bisphosphate; E/N, the ratio of nitrogen contained in RuBP carboxylase to the total leaf nitrogen.

Table I. *Plant Material Used and the Derivation of Modern Wheat*

Genome	Species	Accession No.	Evolutionary Path[a]
A	*Triticum monococcum* L. Einkorn 292	AUS 11429	
	T. monococcum L.	AUS 15194	A ♂
	T. urartu Tum. v nigram	AUS 17974	
	T. boeoticum Boiss. emend Schiem.	KEW C64.146	
			AB ♀
B	*Aegilops speltoides* Tausch	AS1	
		AUS 18940	B ♀
		AUS 18851	
D	*A. squarrosa* L.	G90 CHBS	
		AUS 18890	D ♂
		AUS 18898	
ABD	*T. aestivum* L.	Yecora 70	
		Gabo	ABD
		Highbury	

[a] Refs. 5 and 30.

was measured with a Hartman and Braun absolute IR gas analyzer. Plants were placed in the system for 1 h before measurements were begun. All measurements were made with a PAR flux density at the leaf surface of 1800 μmol quanta m^{-2} s^{-1}, a leaf temperature of 23°C, and leaf to air vapor pressure difference of 13 millibars. Assimilation rate, A, and intercellular p(CO_2), p_i, were calculated using the equations of von Caemmerer and Farquhar (31). The initial slope of A as a function of p_i was found by linear regression of 6 points with p_i less than 100 μbars.

RuBP Carboxylase Assays. *Method A.* About 10 cm^2 of leaf was ground in a Ten Broeck homogenizer in 5 ml of extraction buffer (50 mM Hepes-KOH [pH 7.0], 1 mM Na_2EDTA, 2 mM $MgSO_4$, 10 mM $NaHCO_3$, 10 mM DTT, 1% w/v polyclar) and centrifuged at 2500*g* for 5 min. The supernatant liquid (25 μl) was used for the assay, which was done in triplicate. The assay medium consisted of 100 mM Tricine-KOH (pH 8.1), 20 mM $MgCl_2$, 20 mM $NaH^{14}CO_3$ (18.5 GBq/mol). The final assay volume was 0.5 ml and the assay was done at 30° C in a scintillation vial. After preincubation of the extract for 2 min, the reaction was started by the addition of 1 mM RuBP (Sigma). The assay was stopped after 60 s with 250 μl of 5 N HCOOH and vials were blown dry and counted in a scintillation counter.

Method B. The extraction buffer contained 50 mM Tricine-KOH (pH 8.0), 20 mM $MgCl_2$, 1 mM Na_2EDTA, 10 mM $NaHCO_3$, 50 μM 6-P-gluconate, 10 mM DTT, and 1% w/v polyclar. RuBP (0.5 mM) was generated in the reaction vial 10 min prior to the assay from 0.5 mM ribose-5-P (Sigma) and 5 mM ATP via yeast ribose-5-P isomerase (10 units, Sigma) and ribulose-5-P kinase (10 units, purified from spinach and free of RuBP carboxylase activity). The assay was started by addition of the preactivated enzyme.

[^{14}C]CABP Assay. RuBP carboxylase protein concentration in the crude extracts used for the activity measurements was determined using the radiolabeling technique of Collatz *et al.* (6). This procedure takes advantage of the highly specific tight binding of [2-^{14}C]carboxyarabinatol bisP, CABP, a ^{14}C-labeled analog of the initial product of the carboxylation reaction, to each catalytic site on the RuBP carboxylase enzyme in the presence of Mg^{2+}. Collatz *et al.* (6) demonstrated that about 8 mol CABP was bound to 1 mol of activated RuBP carboxylase, consistent with eight active sites per enzyme molecule. This relationship was found to hold for RuBP carboxylase from all species examined, including the green alga *Chlamydomonas reinhardtii*. Crude extract (25 μl), containing activated RuBP carboxylase, was added to 100 μl of buffer (100 mM Bicine [pH 8.0], 20 mM $MgCl_2$) containing 4 nmol of [2-^{14}C]CABP, and 100 μl of antisera to RuBP carboxylase (from rabbits) was then added. The microfuge tubes were incubated for 2 h at 37°C to precipitate the RuBP carboxylase protein. The precipitate was collected on cellulose acetate filters (pore size, 0.5 μm; Millipore), washed with (0.85% w/v NaCl, 10 mM $MgCl_2$), and the bound ^{14}C determined by liquid scintillation counting. This procedure thus provides a direct and accurate measure of the molar concentration of this enzyme independent of the presence of other proteins in the preparation to be assayed, since one ^{14}C is bound to each catalytic site (6, 14).

In an attempt to alter CABP binding, the activated enzyme was incubated either with or without bicarbonate added to the assay. No difference in CABP binding could be detected. The specificity of this assay was also independent of the antisera used. Normally the assay was conducted with antisera raised against RuBP carboxylase isolated from spinach, but the same amount of RuBP carboxylase was precipitated using antisera which had been raised against RuBP carboxylase isolated from wheat. Similarly, the use of 20% w/v PEG with 60 mM $MgCl_2$ also precipitated the same amount of RuBP carboxylase (see also 14).

Determination of the K_m(CO_2). The K_m(CO_2) of RuBP carboxylase was determined using a mass spectrometer (VG Micromass MM6). Reactions were carried out in a stirred, temperature controlled (30°C) cuvette separated from the mass spectrometer by a plastic membrane. CO_2-free assay buffer (100 mM Bicine [pH 8.2], 20 mM $MgCl_2$, 1 mM EDTA) was bubbled with N_2 or CO_2-free air to produce O_2-free or 21% O_2 concentrations. RuBP concentration in the assay was 0.5 mM using freshly prepared RuBP, as described in method B above. Different CO_2 concentrations were obtained by addition of $NaHCO_3$ to the reaction mixture. The assay was initiated by addition of fully activated enzyme in a crude preparation. The initial linear rate of CO_2 fixation (measured as disappearance of mass 44) was measured at six CO_2 concentrations (100—1800 μbars) for each K_m(CO_2) determination. K_m(CO_2) was calculated using the method of Wilkinson (32).

Nitrogen Analysis. Between 15 and 20 mg of oven-dried leaf material was used for a microKjeldahl digestion. A colorimetric ammonia determination was done with a Technicon autoanalyzer. The nitrogen content of RuBP carboxylase can be calculated from the amino acid analyses (for example, 29) to be about 15% which is close to that generally accepted for protein of 16%. The

ratio E/N was calculated as follows: mg RuBP carboxylase protein per unit leaf area, from the CABP assay, multiplied by 0.15 mg N per mg protein divided by the nitrogen content per unit leaf area.

RESULTS

The relationship between *in vitro* RuBP carboxylase activity and total leaf nitrogen differed between *T. aestivum* and *T. monococcum* (Fig. 1a). The x intercepts were not significantly different from zero so the regressions were constrained to pass through the origin. RuBP carboxylase activity per unit total leaf nitrogen (mmol CO_2 mol N^{-1} s^{-1}) was 1.24 in the hexaploid *T. aestivum* and 0.94 in the diploid *T. monococcum*. This 30% difference was evident over a wide range of leaf nitrogen contents.

The *in vitro* RuBP carboxylase activity and the amount of RuBP carboxylase protein per unit leaf area, measured on the same extract, together with the ratio of the amount of nitrogen

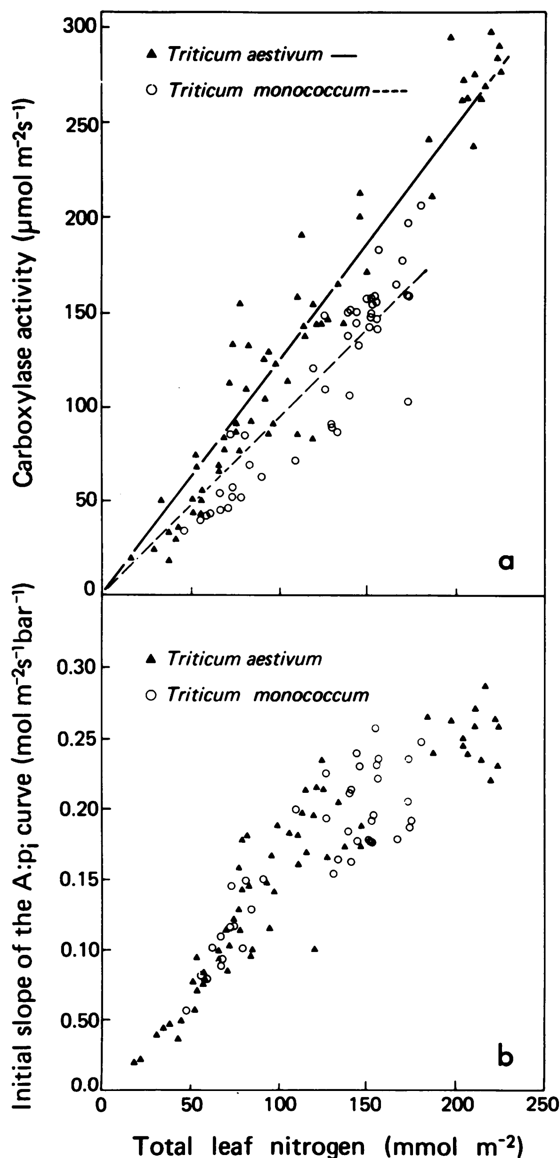

FIG. 1. a, *In vitro* RuBP carboxylase activity (method A, 30°C) *versus* total leaf nitrogen for *T. aestivum* cv Yecora 70 ($y = 1.24N$, $r^2 = 0.98$, $P < 0.001$) and *T. monococcum* ($y = 0.94N$, $r^2 = 0.97$, $P < 0.001$). The *T. aestivum* data are from Evans (9). b, The relationship between the initial slope (23°C) of the curve relating CO_2 assimilation rate, A, to intercellular $p(CO_2)$, p_i, *versus* total leaf nitrogen.

in RuBP carboxylase to the total leaf nitrogen, E/N, are shown for *T. aestivum*, *T. monococcum*, and *T. urartu* (Table II). The specific activity of RuBP carboxylase from *T. aestivum* was 30% higher than that from *T. monococcum*. No significant difference in the ratio E/N (about 21%) was observed between the two genotypes. Using the specific activities from Table II, the ratio E/N can be calculated using the independent data from the regressions in Figure 1a to be 20.5% and 20.9% for *T. aestivum* and *T. monococcum*, respectively. The specific activity of the RuBP carboxylase from *T. urartu*, another diploid A genome, was the same as that of *T. monococcum* and the ratio E/N was also the same. The specific activity of RuBP carboxylase remained constant through senescence in *T. aestivum* while the ratio E/N declined.

Leaves of *T. aestivum* and *T. monococcum* were ground together in three separate experiments (data not shown). In all cases, the specific activity was the average expected from an independent assay of each leaf separately. RuBP carboxylase from *T. aestivum* and *T. monococcum* were also found to have the same pH and Mg^{2+} optima for maximal activity (data not shown). The $K_m(CO_2)$ of the enzymes from *T. aestivum* and *T. monococcum* did not differ significantly, whether determined in N_2 or 21% O_2 (Table III).

A more detailed study of RuBP carboxylase specific activity in relation to genome is presented in Table IV. Three accessions from each of the B, D, and ABD genomes and four from the A genome were used. The specific activity of RuBP carboxylase from three lines of *T. aestivum* was significantly greater than that of the A, B, or D genome species. Little variation in specific activity was found within the A, D, and ABD genomes. Interestingly, while RuBP carboxylase specific activities of the B genome lines were found to be below that of *T. aestivum*, they were still significantly higher than those of the A or D genome species. More variability than had been previously encountered was found in the ratio E/N, particularly in the A genome, which hindered statistical analysis. In some cases, the lower specific activity would have been somewhat offset by greater E/N.

Data for the *in vitro* RuBP carboxylase activity in Table IV were obtained using RuBP generated in the reaction vial just prior to the assay, in contrast to activity measurements presented in Table II and Figures 1a and 3 which were determined using commercially available RuBP. It is not known whether this is the cause of the higher specific activities in Table IV, although it is known that RuBP carboxylase inhibitors form with time in RuBP solutions (21). However, it should be noted that the relative difference in the specific activities between the ABD and A genomes was not altered by assay technique.

The 30% difference in RuBP carboxylase activity per unit of leaf nitrogen between *T. aestivum* and *T. monococcum* (Fig. 1a) was not reflected in photosynthetic characteristics of intact leaves. There was no apparent difference between species in the initial slope of the A:p_i curve for a given nitrogen content (Fig. 1b). The initial slope of the A:p_i curve, or mesophyll conductance, is comprised of two components, namely the CO_2 transfer conductance, g_w, and the carboxylation efficiency, k. The CO_2 transfer conductance is defined as $g_w = A/(p_i - p_c)$, so that the smaller g_w, the greater the fall in the partial pressure of CO_2 between the intercellular spaces and the sites of carboxylation. The carboxylation efficiency is proportional to the rate of RuBP carboxylation at a given p_c. Given that the rate of RuBP carboxylation *in vitro* by *T. aestivum* differs from that of *T. monococcum* (Tables II and IV), it follows that CO_2 transfer conductances for the two wheats must be different because the mesophyll conductances are the same for a given leaf nitrogen content (Fig. 1b).

The curvature in the relationship between the mesophyll conductance and RuBP carboxylase activity has been used to esti-

Table II. *RuBP Carboxylase Activity, Content, Specific Activity and Nitrogen Content Relative to Total Leaf Nitrogen Content from T. aestivum and Two A Genomes*

	n[a]	Activity[b]	Content	Specific Activity[c]	(k_{cat})[d]	E/N[e]
		μmol m⁻² s⁻¹	*g m⁻²*	*μmol mg⁻¹ min⁻¹*	*s⁻¹*	*%*
ABD *Triticum aestivum*						
Young leaf	6	243	3.75	3.89 a		21.3
Senescing leaf	4	72	1.12	3.90 a		13.9
				3.89 ± 0.12	(36)	
A *Triticum monococcum*	7	113	2.42	2.83 b		20.4
A *Triticum urartu*	5	143	2.90	2.97 b		20.6
				2.89 ± 0.08	(27)	

[a] Number of replicates.
[b] *In vitro* RuBP carboxylase activity was determined by method A (30°C).
[c] Values followed by the same letter are not significantly different (P > 0.05). ABD > A, P < 0.001.
[d] mol CO_2 mol enzyme^{-1} s^{-1}, assuming mol wt = 550,000.
[e] The ratio of nitrogen contained in RuBP carboxylase (assumed to be 15% [29]) to the total leaf nitrogen.

Table III. *K_m (CO_2) of RuBP Carboxylase from T. aestivum and T. monococcum*

		n[a]	N_2[b]	n[a]	21% O_2[b]
			μbars		*μbars*
ABD	*T. aestivum* cv Yecora 70	7	470 ± 40a	4	1120 ± 120b
A	*T. monococcum* 292	6	500 ± 60a	5	950 ± 120b

[a] Number of replicates.
[b] Mean ± SE; values followed by the same letter are not significantly different.
[c] To convert μbars to μM, multiply by Henry's constant: 0.0296 at 30°C, *e.g.* 470 μbars = 13.9 μM.

Table IV. *RuBP Carboxylase Parameters from the Different Wheat Genomes*

		n[a]	Content	Specific Activity[b]	(k_{cat})[c]	E/N[d]
			g m⁻²	*μmol mg⁻¹ min⁻¹*	*s⁻¹*	*%*
ABD	*T. aestivum* Yecora 70	7	2.95	5.05		20.8
	Gabo	4	2.57	4.69		21.1
	Highbury	5	2.71	4.80		20.7
				4.88 ± 0.11a	(45)	
A	*T. monococcum* 292	4	2.05	3.62		24.8
	15194	4	1.88	3.75		19.0
	T. urartu	11	2.65	3.74		16.5
	T. boeoticum	4	3.51	3.59		27.1
				3.70 ± 0.06c	(34)	
B	*Aegilops speltoides* AS1	5	2.61	4.40		24.1
	18940	3	3.14	3.69		25.9
	18851	3	3.05	4.20		26.7
				4.16 ± 0.14b	(38)	
D	*A. squarrosa* G90	5	2.57	3.83		21.9
	18890	4	2.63	3.93		24.3
	18898	3	2.41	3.81		23.7
				3.87 ± 0.06c	(35)	

[a] Number of replicates.
[b] *In vitro* RuBP carboxylase activity was determined by method B (30°C). The mean ± SE are presented for each genome, values followed by the same letter are not significantly different (P > 0.05). ABD > A, B, or D, P < 0.001; B > A, P < 0.001; B > D, P < 0.05.
[c] mol CO_2 mol enzyme^{-1} s^{-1}, assuming mol wt = 550,000.
[d] See Table II.

mate the CO_2 transfer conductance for *T. aestivum*, 0.49 mol m^{-2} s^{-1} bar^{-1} (9, 10). The estimate has recently been confirmed by simultaneous measurement of gas exchange and discrimination against ^{13}C. The discrimination is a function of both the ratio p_i/p_a and p_c/p_a. From Farquhar *et al.* (12), $r = (p_i - p_c)/p_a = A/(g_w \cdot p_a)$. Since $r = [\delta - \delta_{atm} + a + (b - a)p_i/p_a]/b$, (equation 16, [12]), $g_w = A \cdot b/[p_a(\delta - \delta_{atm} + a + (b\text{-}a)p_i/p_a)]$, where a and b are the fractionations associated with diffusion in air and RuBP carboxylation, respectively (constants), and A, p_i/p_a and $(\delta - \delta_{atm})$, the $^{13}CO_2$ enrichment, are measured. The CO_2 transfer conductances measured with this method were 0.46 ± 0.07 and 0.64 ± 0.02 mol m^{-2} s^{-1} bar^{-1} for *T. aestivum* and *T. monococcum*, respectively (10; Evans, Sharkey, and Berry, in preparation).

Using these estimates of g_w, the gas exchange data in Figure 1b have been reexpressed as carboxylation efficiencies (the initial slope of the curve relating assimilation rate to the p(CO_2) at the site of carboxylation). The effects of the difference in specific activity of RuBP carboxylase now become apparent. The difference in the slopes of the two lines in Figure 2 is similar to Figure 1a, and the carboxylation efficiency in the intact leaf is directly proportional to the *in vitro* carboxylase activity (Fig. 3). The regressions in Figure 3 for *T. aestivum* and *T. monococcum* were not significantly different: carboxylation efficiency (mol m^{-2} s^{-1} bar^{-1}) = 0.00205·RuBP carboxylase activity (μmol m^{-2} s^{-1}).

DISCUSSION

There have been two reports describing differences in RuBP carboxylase associated with ploidy. In a comparison between hexaploid and decaploid tall fescue, it was reported that both assimilation rate and the specific activity of RuBP carboxylase increased with ploidy (23). This was subsequently shown to be an artefact, due to assaying the enzyme without activation (16). In a comparison between RuBP carboxylase from isogenic diploid and tetraploid ryegrasses, differences in the $K_m(CO_2)$ and isoelectric point were found, while specific activity remained constant (13). Two groups of workers failed to reproduce these findings. They found that RuBP carboxylase from the two ploidies had the same $K_m(CO_2)$, the same isoelectric point, and constituted a similar proportion of the soluble protein in both cases (18, 24). A study of an isogenic ploidy series in alfalfa also revealed no differences in specific activity, K_m, or carboxylase/oxygenase ratio (19), nor in the proportion of soluble protein that RuBP carboxylase constituted (20). One would expect a ploidy series to have identical RuBP carboxylase kinetics if the material was isogenic. The effects of ploidy are more likely to be manifested in anatomical changes.

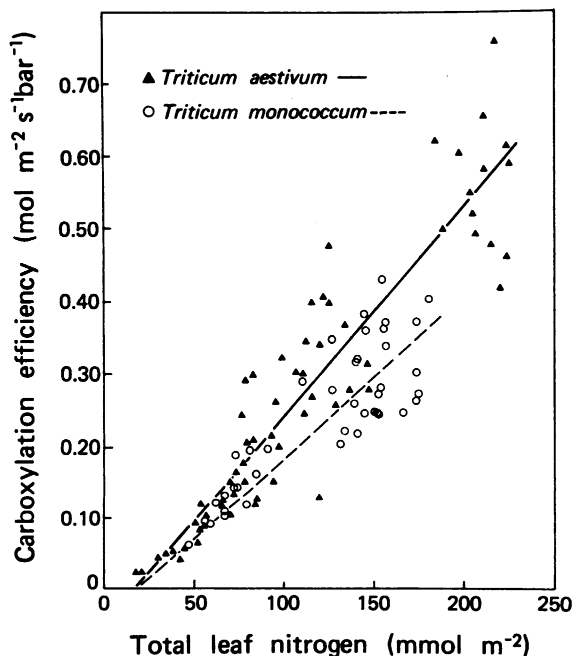

FIG. 2. Carboxylation efficiency, k, *versus* total leaf nitrogen. *T. aestivum*, $g_w = 0.46$, $y = 0.00289N - 0.047$, $r^2 = 0.86$, $P < 0.001$. *T. monococcum*, $g_w = 0.64$, $y = 0.00222N - 0.036$, $r^2 = 0.95$, $P < 0.001$.

Studies which have compared the kinetic characteristics of RuBP carboxylase from different species have revealed more consistent patterns. Jordan and Ogren (15) have shown that there have been changes in the $K_m(CO_2)$ and the carboxylase/oxygenase ratio in markedly different autotrophic organisms through evolution. Variation between C_3 and C_4 plants in the $K_m(CO_2)$ has been shown in other surveys (33) and in the specific activity by Seemann *et al.* (27). Seemann and Berry (28) found differences in the specific activities but not the $K_m(CO_2)$ of RuBP carboxylase extracted from spinach and soybean, and this agreed with observed differences in photosynthetic rate.

RuBP Carboxylase Specific Activity. The implication of Figure 1a is either that the two wheat genotypes have RuBP carboxylase with different specific activities, or that the proportions of total leaf nitrogen in RuBP carboxylase, E/N, differ. Table II shows that the first is true; the ratios E/N are the same. If E/N is taken as 21%, the regression lines in Figure 1a predict specific activities of 3.80 and 2.88 for *T. aestivum* and *T. monococcum*, respectively, in agreement with Table II. The finding is supported by other work. In a study of polyploid series in wheat, Dean and Leech (7) observed no change in the proportion of carboxylase protein to soluble protein. RuBP carboxylase from *T. aestivum* and *T. monococcum* averaged around 40% of the soluble protein, or about 20% of the total leaf nitrogen. Similarly, in a polyploid study of alfalfa, RuBP carboxylase represented 44% of soluble protein (20). Variability in the ratio E/N in Figure 1a and Tables II and IV is probably due to the use of leaves of different rank and age. Possession of RuBP carboxylase enzyme with a lower specific activity could be offset by an increase in the ratio E/N, but not without reducing other processes which may also be limited by nitrogen.

The specific activities in Tables II and IV are comparable to other published values if they are brought to the same temperature using the Q_{10} of the enzyme (2); 4.3, 4.3, 3.9, 3.7, 3.2, 2.8 μmol mg^{-1} min^{-1} (30°C) (4, 28, 27, 27, 6, 14, respectively). These specific activities are higher than those frequently seen for purified enzyme but are routinely obtained by rapidly assaying the crude extract, ensuring that the enzyme is fully activated and that the RuBP is free of xylulose bisphosphate (21).

RuBP Carboxylase Activity and Leaf Gas Exchange. It was a surprise that the initial slopes of the A:p_i curves were the same for *T. aestivum* and *T. monococcum* for the same nitrogen contents (Fig. 1b), given that the two species had different carboxylase activities. That the initial slope of the A:p_i curve is a function of the *in vivo* RuBP carboxylase activity is supported by several studies (9, 28, 31). Published data, however, show curvature in the relationship between initial slope of the A:p_i curve and *in vitro* RuBP carboxylase activity (9, 31). This curvature is likely to be the consequence of a resistance to CO_2 transfer from the intercellular spaces to the site of carboxylation. Previously, it has been impossible to determine the magnitude of the CO_2 transfer conductance experimentally. Two independent methods have been used to obtain the CO_2 transfer conductance for *T. aestivum*, and these are in good agreement.

Evans (9) estimated g_w from a double reciprocal plot of mesophyll conductance *versus in vitro* RuBP carboxylase activity. This assumed that the interaction between the transfer conductance and CO_2 assimilation rate was small. It also assumed that all the replicates have the same g_w, which may result in a small

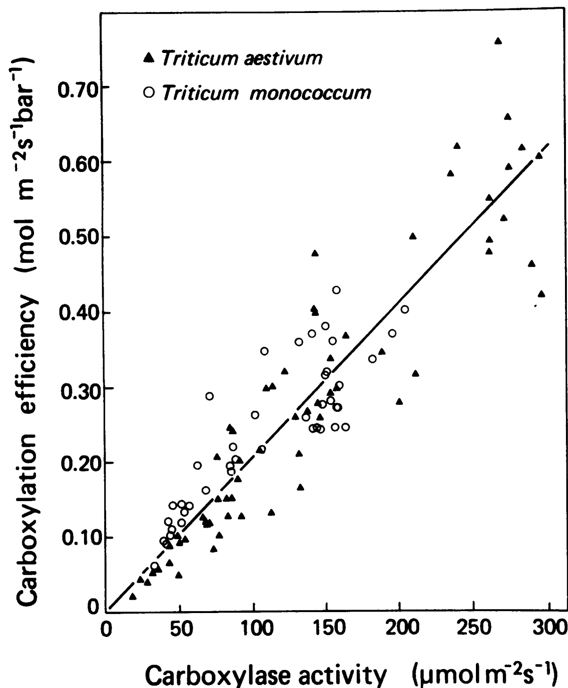

FIG. 3. Carboxylation efficiency (23°C), k, *versus in vitro* RuBP carboxylase activity. *T. aestivum*, $g_w = 0.46$ (data from 9); *T. monococcum*, $g_w = 0.64$; $k = 0.00205x$, $r^2 = 0.96$, $P < 0.001$.

overestimate of the transfer conductance. When g_w was calculated for *T. aestivum* and *T. monococcum* from discrimination against $^{13}CO_2$, the fractionation by RuBP carboxylase was taken as 27‰ (10). If this constant is actually larger, then again, g_w would be overestimated. However, the difference between the two wheats would remain. The present study shows that differences in the conductance to CO_2 through the cell walls and liquid phase are as important as those in RuBP carboxylase specific activity in influencing the rate of CO_2 assimilation in *T. aestivum* and *T. monococcum*.

The greater conductance to CO_2 through the cell walls and liquid phase in *T. monococcum* may be due to a greater area, per unit leaf area, of mesophyll cell wall exposed to intercellular air space, A^{mes}/A. Sasahara (26) found A^{mes}/A to be 19.6 ± 0.9 in *T. boeoticum* and *T. monococcum* and 12.7 ± 0.4 in *T. aestivum*. Parker and Ford (22) obtained 9.8 in *T. monococcum*, 15.3 in *T. urartu*, and 10.5 in *T. aestivum*. The measurement of A^{mes}/A is difficult and presumably there is variation due both to accession and to growth conditions. Nevertheless, the results quoted suggest that A^{mes}/A in the diploid A genome exceeds that in *T. aestivum* by 20 to 50%. This seems to agree with the measured difference in the conductance to CO_2 through the cell walls and liquid phase. However, the comparison is valid only if the chloroplasts effectively cover all of A^{mes}, which is presumably not the case as the leaf ages. Other factors, such as chloroplast thickness and carbonic anhydrase activity, may also contribute to the difference.

The carboxylation efficiency, k, is the initial slope of the curve relating assimilation rate to $p(CO_2)$ at the site of carboxylation. The carboxylation efficiency can be calculated from the initial slope of the A:p_i curve if the conductance to CO_2 through the cell walls and liquid phase is known (9). It is apparent that carboxylation efficiency is proportional to leaf nitrogen in the same way that *in vitro* RuBP carboxylase activity is (Fig. 2). The fact that the carboxylation efficiencies for *T. aestivum* and *T. monococcum* superimpose when expressed against *in vitro* RuBP carboxylase activity (Fig. 3) suggests that the $K_m(CO_2)$ is the same for the two enzymes. This indeed appears to be the case (Table III). The $K_m(CO_2)$ in N_2 are comparable with other wheat data (33).

According to the analysis of von Caemmerer and Farquhar (31), providing RuBP carboxylase is sufficiently activated, the dependence of CO_2 assimilation rate, A, on p_c, dA/dp_c, in the presence of oxygen at Γ is, $k = V_{max}/[\Gamma + K_c(1 + O/K_o)]$, where V_{max} is the maximum catalytic rate of the enzyme, Γ is the CO_2 compensation point in the absence of dark respiration (30 μbars), K_c (250 μbars) and K_o (190 mbars) are the Michaelis constants for CO_2 and O_2 at 23°C, and O is the $p(O_2)$ (200 mbars). V_{max} at 23°C = $[V(30°C)/(\log 7 \cdot 2.2)] \cdot 1.1$, where 2.2 is the Q_{10} of RuBP carboxylase (2), and 1.1 is to obtain V_{max} by extrapolation of the double reciprocal plot used to obtain the Michaelis constants. Thus, the *in vivo* carboxylation efficiency $k = 0.0011V(30°C)$. The slope of the regression line from Figure 3 was nearly double this, implying that the *in vitro* RuBP carboxylase activity was insufficient to account for the observed *in vivo* rate. However, if a correction is made to include the higher specific activity determined from method B, $k = 0.0020 \times$ (36, method A)/(45, method B) = $0.0016V(30°C)$). The *in vitro* RuBP carboxylase activity still needs to be 30% greater to account for the observed *in vitro* rate given the values of g_w, $K_m(CO_2)$, and Γ used above.

High Specific Activity Associated with the B Genome. The RuBP carboxylase enzyme consists of two types of subunits. The small subunit is coded on nDNA, while the large subunit is coded on the ctDNA (5). Because the chloroplast genome is maternally linked, it has been possible to derive the path that hybridization occurred in the formation of *T. aestivum*. Chen *et al.* (5) found that the isoelectric point of the small subunit was the same for all the wheat genomes. However, the isoelectric point for the large subunit from the A and D genomes differed from that of the B and ABD genomes. They proposed that *T. monococcum* (♂) A, combined with *A. speltoides (♀) B*, to form *T. dicoccum* and that *T. dicoccum* (♀) AB, then combined with *A. squarrosa* (♂) D, to form *T. aestivum* ABD, as indicated in Table I. This path was confirmed from DNA analysis by Vedel *et al.* (30). The results in Table IV show that the B genome is correlated with the higher specific activity, as both the ABD and B accessions had significantly higher specific activities than the A and D accessions. This suggests that the higher specific activity of the enzyme is associated with the presence of the B genome large subunit of carboxylase. That the B accessions had lower specific activities than those of *T. aestivum* may indicate that the contemporary *A. speltoides* accessions differ from that which gave rise to *T. dicoccum*, a view supported by the DNA analysis of Vedel *et al.* (30).

Conclusion. RuBP carboxylase constitutes about 20% of total leaf nitrogen, making it the largest nitrogen containing component in a leaf. At the same time, this enzyme may still be rate-limiting to photosynthesis under many conditions. These facts are the basis for considerable scientific interest, at many levels, in the kinetic properties of RuBP carboxylase. Our interest is to attempt to relate the biochemical characteristics of a leaf to its photosynthetic performance. At some point our knowledge clearly falls short, as our calculations of photosynthesis demonstrate. However, we are able to observe consistent patterns of variation at both the *in vivo* and *in vitro* level. We have also included a consideration of the transfer resistance to CO_2 diffusion to the site of carboxylation. There have been few attempts to quantitatively relate photosynthetic performance and leaf biochemistry (9, 28, 31), but we feel it is essential if genetically based variation in the kinetic properties of RuBP carboxylase is to be uncovered and confirmed. Such variation can provide a starting point for attempts to alter the kinetic properties of this enzyme. Our findings may lead to the identification of changes in the RuBP carboxylase enzyme which have led to altered

specific activities.

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