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Carbon Dioxide Fixation by Detached Cereal Caryopses[1]

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ABSTRACT

Immature detached cereal caryopses from barley (*Hordeum vulgare* L. var distichum cv Midas) and wheat (*Triticum aestivum* L. cv Sicco) were shown to be capable of fixing externally supplied $^{14}CO_2$ in the light or dark. Green cross cells and the testa contained the majority of the ^{14}C-labeled material. Some ^{14}C-labeled material was also found in the outer, or transparent, layer and in the endosperm/embryo fraction. More ^{14}C was recovered from caryopses when they were incubated in $^{14}CO_2$ without the transparent layer, thus suggesting that this layer is a barrier to the uptake of CO_2. In all cases, significant amounts of ^{14}C-labeled material were found in caryopses after dark incubation with $^{14}CO_2$. Interestingly, CO_2 fixation in the chlorophyll-less mutant *Albino lemma* was significantly greater in the light than in the dark. The results indicate that intact caryopses have the ability to translocate ^{14}C-labeled assimilate derived from external CO_2 to the endosperm/embryo. Carboxylating activity in the transparent layer appears to be confined to phosphoenolpyruvate carboxylase activity but that in the chloroplast-containing cross-cells may be accounted for by both ribulose-1,5-bisphosphate carboxylase-oxygenase and phosphoenolpyruvate carboxylase activity. Depending on a number of assumptions, the amount of CO_2 fixed is sufficient to account for about 2% of the weight of starch found in the mature caryopsis.

The main source of assimilates for grain-filling in cereals is thought to be photosynthesis by the ear, stem, and flag leaf after pollination. In barley, and possibly other cereals, material stored in the stem before pollination may make a significant contribution to final grain yield (3). The proportion of total photosynthetic activity met by ear photosynthesis depends on a number of factors, including species, cultivar, environmental conditions, developmental stage and methods used in the determination of rates of ear photosynthesis. Thus, estimates of the contribution made by ear photosynthesis to final grain weight varies between as little as 13% and as much as 76% (8). Certainly, all the green tissues of the wheat ear are capable of photosynthesis (10) and therefore may be potential sources of assimilate for grain filling.

The Chl-containing cells of the immature pericarp of barley, wheat, rye, rice, and oats are the site of caryopsis photosynthesis (8). These form a continuous band (known as the green layer) on the outside of the testa and in turn are covered by a layer of nonchlorenchymatous cells (known as the transparent layer). Together these two layers constitute the pericarp. The Chl-containing cells of the barley caryopsis are capable of high rates of light-dependent oxygen evolution (6), and there is evidence to suggest that in oats and wheat as well as in barley, these cells have some capacity for C_4 metabolism (11, 12). In barley pericarps for example, the first-formed product of photosynthesis is the C_4-acid malate which is subsequently rapidly converted to sucrose (11).

Evans and Rawson (10) have found by infrared gas analysis that wheat grains can assimilate CO_2 at rates almost equal to rates of dark respiration until the late stages of grain development. However, we do not know whether or not atmospheric CO_2 fixed by the caryopsis can be translocated to the endosperm/embryo. Nor do we know what contribution pericarp photosynthesis may make to the overall economy of grain-filling. The present work is an attempt to answer these questions.

MATERIALS AND METHODS

Plant Material. Barley (*Hordeum vulgare* L. var hexastichum *Albino lemma*, and *Hordeum vulgare* L. var distichum cv Midas) and wheat (*Triticum* aestivum L. cv Sicco) were grown from seed in pots of 180 mm diameter. Six plants per pot were grown in Levington's peat-based potting compost (Fisons Ltd, Horticulture Division, Bramford, Ipswich, UK) under glasshouse conditions. Natural daylength was extended to 20 h with 400 W mercury vapor lamps and the mean daily temperature was 18°C. The ages of the barley cv Midas caryopses were determined using a developmental time scale of 60 'days' from anthesis to harvest-ripeness (5). Wheat caryopses were aged using a similar developmental time scale based on field-grown wheat harvested in 1981. *A. lemma* is a six-row barley and is a chemically induced mutant. The pericarp, lemma, and palea of the grain are albino with the exception of small green areas at the tips of the lemma and palea. However, the awns, sterile glumes, and remainder of the plant contain Chl, and the plant grows well in a glasshouse after vernalization. The age of each ear of *A. lemma* was estimated by ageing an ear of cv Midas which reached anthesis on the same day as that of the *A. lemma* ear. In this way, comparison can be made at similar stages of development in caryopses from different species. All caryopses were 25 d after anthesis since at this stage Chl content and grain growth rates are maximal (6).

$^{14}CO_2$ Fixation by Detached Caryopses. The method used was a modification of the method of Nutbeam and Duffus (11). The sterile glumes, lemma, and palea were dissected from 4 grains, and the caryopses were placed on filter paper discs previously soaked in 330 mM sorbitol, 50 mM Tricine-KOH (pH 7.5). In a number of experiments, the transparent layer of the pericarp was also removed. The detached caryopses on moist discs were placed in an airtight Perspex chamber (volume 11 cm^3) and 0.02 cm^3 of 17 mM sodium [^{14}C]carbonate (specific activity 2.16×10^6 Bq mol^{-1}) was injected through a rubber seal into a well containing 0.15 cm^3 of 13 M lactic acid. The final concentration of CO_2 in the chamber was 0.1% by volume.

The chamber was illuminated with a 1000 W tunsten halogen lamp (photon flux density in the chamber was 710 μmol m^{-2} s^{-1}) for varying periods of time. A Perspex box (dimensions 250 × 250 × 60 mm) with cold water circulating through it was positioned between the lamp and the chamber to maintain the chamber at 21°C. The light-dependence of $^{14}CO_2$ fixation was

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investigated by covering the chamber with aluminum foil. The conditions for the dark experiments were as described for investigations in the light. Photon flux density did not limit CO_2 fixation.

Extraction of ^{14}C-Labeled Material. Following incubation with $^{14}CO_2$, the caryopses were rinsed quickly in distilled water, and the transparent layer was dissected from the caryopsis using fine tweezers. The cross-cells, testa, and nucellar epidermic (inner layers) were removed together as these tissues are difficult to separate from caryopses aged 25 d after anthesis. Similarly, the embryo was extracted with the endosperm as the embryo is very small at this stage of caryopsis development. The tissues were homogenized in 80% (v/v) ethanol at 70°C with hand-held all-glass tissue grinders. Each homogenate was washed twice with two aliquots of hot ethanol, and the washings plus ethanol-insoluble material were centrifuged for 2 min at 700*g*. The supernatants were made up to 10 cm^3, and the amount of ^{14}C was determined in 0.1 cm^3 samples by scintillation counting. The ethanol-insoluble residue was mixed with 5 cm^3 distilled water and left for 24 h at room temperature. After centrifugation at 700*g* for 2 min, ^{14}C was determined in replicate 0.1 cm^3 samples. The results for amounts of ^{14}C recovered as soluble material represent the sum of the ^{14}C recovered in the ethanol-soluble and water-soluble extracts.

Water-insoluble material was hydrolyzed by adding 2 cm^3 of 2 M HCl to the twice washed residue and then heating for 2 h at 100°C. A drop of the solution was added to I_2/KI to check that the starch had been hydrolyzed. All measurements of radioactivity were corrected to disintegrations per min (dpm) using quench curves, and the amounts of C fixed were recorded as pmol $tissue^{-1}$ over the complete incubation period using the initial value for specific activity of $^{14}CO_2$ in the assimilation chamber.

Enzyme Extraction and Assay. Between 20 to 30 grains were removed from the middle of 4 ears of cv Midas or *A. lemma* and either the transparent layer or inner layers dissected from the caryopses. Grains aged about 25 d after anthesis were used. The tissues were homogenized in a hand-held all-glass tissue grinder in 2 cm^3 of ice-cold isolation medium (1 mM $MnCl_2$, 1 mM $MgCl_2$, 10 mM mercaptoethanol, 330 mM sorbitol, 50 mM Tricine [pH 7.5]). The extract was centrifuged (400*g*, 2 min), and the pellet was reextracted with 1 cm^3 isolation medium. The extracts and pellet were recombined and centrifuged as before, and the supernatant was used as the enzyme extract. For Rubisco[2] a modification of the assay used by Bahr and Jensen (2) was used. For enzyme activation, tubes containing 1 cm^3 incubation medium (25 mM $MgCl_2$, 10 mM mercaptoethanol, 25 mM Hepes [pH 7.8], 19.8 mM $NaHCO_3$, 5.18×10^5 Bq of $NaH^{14}CO_3$ [specific activity 2.15×10^6 Bq mol^{-1}]) and 0.1 cm^3 emzyme extract were placed in a water bath at 25°C for 4 min. The photon flux density was 700 μmol m^{-2} s^{-1}. To initiate the carboxylase reaction, 0.1 cm^3 3.6 mM ribose-5-phosphate and 3.6 mM ATP in the incubation buffer were added. Immediately after mixing and at 5 and 10 min intervals, 0.1 cm^3 was withdrawn and mixed with 0.5 cm^3 5% (W/V) TCA in ethanol. The ethanolic mixtures were heated at 70°C for 15 min to drive off unfixed $^{14}CO_2$. Samples taken before and after heating at 70°C were counted to ensure that all unfixed $^{14}CO_2$ was driven off. After 15 min, the ^{14}C-level in each tube remained constant.

The amount of ^{14}C was determined in 0.1 cm^3 samples using scintillation counting. Counting efficiency was 80%. Values obtained for controls containing either substrate or enzyme were subtracted from those obtained with both enzyme and substrate present. The assays were replicated three times. The linearity of the reaction rate was confirmed by sampling at 1 min intervals for 10 min. For PEPC, 0.1 cm^3 samples of enzyme extract were mixed with 1 cm^3 of the incubation medium and 0.1 cm^3 of 80 mM sodium glutamate in incubation buffer was added. After equilibration (5 min, 25°C) the reaction was initiated by the addition of 0.1 cm^3 33 mM PEPC in incubation buffer. Photon flux density was 700 μmol m^{-2} s^{-1}. Immediately after mixing and at 5 and 10 min intervals, 0.1 cm^3 samples were mixed with 0.5 cm^3 5% TCA in ethanol and heated at 70°C for 15 min, and the acid-stable ^{14}C-labeled compounds were counted for activity. Controls were as described above, and again the linearity of the reaction rate was confirmed by sampling at 1 min intervals for 10 min. Total Chl content of the inner layers extract was determined by the method of Arnon (1).

RESULTS

$^{14}CO_2$ Fixation by Barley (cv Midas) Caryopses. The amount of ^{14}C found in caryopses after $^{14}CO_2$ incubation in the light increased logarithmically over the 15 min period (Table I). By far the greatest proportion of radioactivity recovered was found in the soluble fraction extracted from the inner layers. Since the chloroplast-containing cross-cells comprise the major part of the inner layer fraction, it is assumed in this work that the dpm present are principally in the cross-cells and that it is these cells that are mainly responsible for the observed carbon dixoide fixation. Significant amounts of radioactivity were also found in the soluble fraction of the transparent layer. Amounts of label found in the soluble fraction derived from the endosperm/embryo were small and increased slightly over the period of incubation. Very little label was found in the insoluble fraction. Amounts were detectable only by 15 min after the start of the incubation and were present only in the transparent layer and cross-cells.

In the dark, the amounts of $^{14}CO_2$ incorporated were small. At 10 min, all the label was found in the soluble fraction of the transparent layer, but by 15 min amounts were more evenly distributed between the tissues analyzed. Label was also found in the insoluble fractions isolated from the transparent layer, cross-cells, and endosperm/embryo.

When isolated transparent layers of barley (cv Midas) caryopses were incubated for 15 min in $^{14}CO_2$, considerable amounts of label were found in the insoluble fraction (Table II). Radioactivity was also detectable in the insoluble fraction. The amounts of $^{14}CO_2$ fixed by caryopses were much greater when the transparent layer was removed. As before, the greatest amount of label was found in the soluble fraction of the cross-cells. At both 10 and 15 min, there was a considerable incorporation of $^{14}CO_2$ into the insoluble fraction of the cross-cells. $^{14}CO_2$ incorporation into the insoluble fraction of the endosperm/embryo was very much less.

In the absence of the transparent layer, considerable amounts of $^{14}CO_2$ were incorporated into caryopses in the dark. Label was found only in the soluble fractions, of which 32% was present in the endosperm/embryo.

Distribution of ^{14}C in Chl-less Caryopses of Barley (*A. lemma*). When intact caryopses were incubated in the light for 10 min in $^{14}CO_2$, more than half the radioactivity was found in the transparent layer (Table III). A substantial proportion of the total ^{14}C recovered (30%) was present in the endosperm/embryo fraction. No label was found in the insoluble fractions. In the absence of the transparent layer, considerably more $^{14}CO_2$ was incorporated in the light, with the proportion increasing in the inner layers and increasingly markedly in the endosperm/embryo. Very small amounts of label were found in the insoluble fraction. $^{14}CO_2$ fixation in intact *A. lemma* caryopses after a 10 min incubation in the dark was more than half of that observed in the light. After 15 min the amount incorporated had doubled but was still present only in the soluble fraction. The distribution between the different tissues was similar, with the transparent layer con-

[2] Abbreviations: Rubisco, ribulose-1,5-bisphosphate carboxylase-oxygenase; PEPC, phosphoenolpyruvate carboxylase.

Table I. *$^{14}CO_2$ Fixation by Intact Barley (cv Midas) Caryopses in Light and Dark*

Results are the mean ± SD of three separate experiments, except as noted. Caryopses aged 25 d after anthesis were incubated with 0.1% $^{14}CO_2$ for different times, and the ^{14}C-labeled material was extracted in hot 80% ethanol followed by water after dissection of the separate tissue fractions. Results are expressed in pmol C fixed per caryopsis and as percent of total ^{14}C (as dpm) recovered.

Tissue	Incubation Time	^{14}C Recovered per Tissue			
		Soluble		Insoluble	
	min	*pmol*	*%*	*pmol*	*%*
Incubations in light					
Transparent layer	2.5[a]	1.25	19	0	0
Inner layers		5.75	78	0	0
Endosperm		0.25	3	0	0
Transparent layer	5[a]	0	17	0	0
Inner layers		35.5	73	2.5	5
Endosperm		2.5	5	0	0
Transparent layer	10	29.8 ± 4.6	19	0	0
Inner layers		111.1 ± 0.2	74	5 ± 5	2
Endosperm		7.0 ± 0.8	5	0	0
Transparent layer	15	61.5 ± 29.5	6	2.2 ± 1.1	+[b]
Inner layers		892.0 ± 41.4	79	30 ± 9.1	2
Endosperm		140.0 ± 10.5	12	1.1 ± 1.1	+[b]
Incubations in dark					
Transparent layer	10	1.67 ± 0.44	100	0	0
Inner layers		0	0	0	0
Endosperm		0	0	0	0
Transparent layer	15	2.66 ± 1.33	28	0.55 ± 0.33	6
Inner layers		2.22 ± 0.33	24	0.55 ± 0.33	6
Endosperm		2.33 ± 1.11	25	1.0 ± 0.22	11

[a] Averages of two separate experiments. [b] + = <1%.

Table II. *$^{14}CO_2$ Fixation by the Transparent Layer and by Caryopses Minus the Transparent Layer in Light and Dark in Barley cv Midas*

Results are the mean ± SD of three separate experiments, except as noted. Tissues were labeled with $^{14}CO_2$ and the ^{14}C-labeled material extracted as described in Table I.

Tissue	Incubation Time	^{14}C Recovered per Tissue			
		Soluble		Insoluble	
	min	*pmol*	*%*	*pmol*	*%*
Incubations in light					
Transparent layer	15	33.7 ± 3.9	98	0.77 ± 0.11	2
Caryopses minus transparent layer					
Inner layers	10	887.0 ± 1.1	83	53.5 ± 21.3	4
Endosperm		125.0 ± 28.8	12	4.21 ± 3.2	+[a]
Inner layers	15	2497.0 ± 60.9	85	184.7 ± 79.1	6
Endosperm		243.3 ± 12.5	8	5.9 ± 2.9	+[a]
Incubation in dark					
Inner layers	10[a]	37.5	68	0	0
Endosperm		17.7	32	0	0

[a] + = <1%. [b] Averages of two separate experiments.

taining almost half the total ^{14}C recovered. $^{14}CO_2$ incorporation in the dark was doubled following removal of the transparent layer. Under these conditions, 67% of total dpm recovered was present in the endosperm/embryo.

Distribution of ^{14}C in Wheat (cv Sicco) Caryopses. Following a 15 min incubation of intact wheat caryopses with $^{14}CO_2$ in the light, almost half the label recovered was present in the soluble fraction of the transparent layer (Table IV). The soluble fraction from the cross-cells and endosperm/embryo accounted for the greater part of the remainder with the major proportion of this being present in the cross-cells. Small amounts of label were present in the insoluble fraction of all tissues. $^{14}CO_2$ incorporation increased upon removal of the transparent layer. The proportion present in the soluble fraction of the cross-cells did not change greatly but the amount present in the insoluble fraction rose to over a third of the total radioactivity recovered. The proportion of total label recovered also increased in the endosperm/embryo with a small amount present in the insoluble fraction.

Rubisco and PEPC in the Outer Layers of Caryopses. Rubisco activity was not detected in the transparent layers of barley cv Midas (Table V) or wheat cv Sicco (not shown). Activity was not determined for *A. lemma*. Significant Rubisco activity was detected in the cross-cells of barley cv. Midas but none was detectable in the cross-cells of *A. lemma*. The Rubisco assay was checked using flag leaf extracts from wheat cv Sicco, which had

Table III. *$^{14}CO_2$ Fixation in Caryopses of Barley (A. lemma) in Light and Dark*

Results are the mean ± SD of three separate experiments, except as noted. Caryopses aged 25 d after anthesis were incubated both with and without the transparent layer in $^{14}CO_2$ and the ^{14}C-labeled material extracted as described in Table I.

Tissue	Incubation Time (*min*)	^{14}C Recovered per Tissue			
		Soluble		Insoluble	
	min	*pmol*	*%*	*pmol*	*%*
Incubations in the light					
Intact caryopses					
Transparent layer	10	36.7 ± 8.9	53	0	0
Inner layers		11.8 ± 3.9	17	0	0
Endosperm		21.2 ± 4.3	30	0	0
Caryopses minus transparent layer					
Inner layers	10	120.9 ± 30.7	41	3.2 ± 2.1	1
Endosperm		173.6 ± 35.7	58	0	0
Inner layers	20	95.6 ± 4.2	28	0	0
Endosperm		241.8 ± 10.5	71	1.3 ± 1.0	1
Incubations in the dark					
Intact caryopses					
Transparent layer	10[a]	15.8	44	0	0
Inner layers		8.1	23	0	0
Endosperm		12.8	23	0	0
Transparent layer	15	39.4 ± 10.5	47	0	0
Inner layers		20.5 ± 7.0	24	0	0
Endosperm		24.4 ± 1.3	29	0	0
Caryopses minus transparent layer					
Inner layers	15	46.5 ± 10.2	32	0.44 ± 0.33	+[b]
Endosperm		98.7 ± 12.8	67	0.33 ± 0.11	+[b]

[a] Averages of two separate experiments. [b] + = <1%.

Table IV. *$^{14}CO_2$ Fixation by Intact Wheat (cv Sicco) Caryopses and by Caryopses minus the Transparent Layer in the Light*

Results are the mean ± SD of three separate experiments. Tissues were labeled with $^{14}CO_2$, and the ^{14}C-labeled material was extracted as described in Table I. Incubation time was 15 min. Caryopses were aged 25 d after anthesis.

Tissue	^{14}C Recovered per Tissue			
	Soluble		Insoluble	
	pmol	*%*	*pmol*	*%*
Intact Caryopses				
Transparent layer	598 ± 47.4	47	1.8 ± 0.55	+[a]
Inner layers	522 ± 13.1	41	21.9 ± 1.8	2
Endosperm	128 ± 4.1	10	0.44 ± 0.11	+[a]
Caryopses minus transparent layer				
Inner layers	963 ± 51.5	45	784 ± 164	37
Endosperm	358 ± 7.2	17	17.6 ± 2.0	1

[a] + = <1%.

a Rubisco activity of 2.9 ± 0.4 μmol CO_2 min^{-1} mg Chl^{-1}. PEPC activity was present in transparent layers and cross-cells of both cv Midas and *A. lemma*. The activity in the transparent layer of *A. lemma* was about three times greater than that in cv Midas, whereas activity in the cross-cells of *A. lemma* was about half that of cv Midas.

DISCUSSION

The results show that even at short exposure times, intact cereal caryopses, with or without Chl in the pericarp, can fix externally supplied CO_2 both in the light and dark.

The results also show that, in barley cv Midas and *A. lemma* and in wheat cv Sicco, label derived from $^{14}CO_2$ fixed in the light or dark can be detected in the transparent layers, cross-cells, and endosperm/embryo, thus indicating that carbon can be transferred from the atmosphere to the interior of the grain. The amount of CO_2 fixed by both wheat and barley caryopses in 15 min is very similar. However, the distribution within the caryopses is rather different, with more than 73% of the total being present in inner layers of barley but only around 40% in wheat. The reason for this is unknown but may be related to the observation (7) that the cross-cell layer in wheat is only one cell thick but in barley is two to three cells thick. Thus, while the wheat cross-cell layer fixes as much CO_2 as barley, it may not have the same capacity for storage of assimilate and excess may

Table V. *Rubisco and PEPC Activity in Transparent Layers and Inner Layers of Caryopses of Barley (cv. Midas and A. lemma)*

Results are the mean ± SD of three separate determinations, except as noted. Between 20 to 30 caryopses aged 25 d after anthesis were removed from the middle of 4 ears, and the transparent layers and inner layers were dissected out. Following activation, Rubisco activity was determined in tissue extracts by the substrate-dependent incorporation ^{14}C from NaH $^{14}CO_3$ into an acid-stable fraction. PEPC activity was determined by the substrate-dependent incorporation of ^{14}C from NaH $^{14}CO_3$ into an acid-stable fraction.

Enzyme	Caryopsis Layer		
	Transparent layer	Inner layers	Inner layers
	nmol CO_2 min^{-1} $tissue^{-1}$		*nmol CO_2 min^{-1} mg Chl^{-1}*
Barley (cv Midas)			
Rubisco	0	0.1 ± 0.01	8.7 ± 0.9
PEPC	4.2 ± 0.7	140.0 ± 1.2	2899 ± 151
Barley (*A. lemma*)			
Rubisco	ND[a]	0[b]	
PEPC	11.5 ± 2.2	6.1 ± 0.6	

[a] Not determined. [b] Average of two determinations.

be transferred to the transparent layer.

It is not clear what proportion of the labeled material present in the transparent layer is derived from fixation of CO_2 *in situ* and what proportion is from CO_2 fixation in cross-cell photosynthesis and subsequent translocation to the transparent layer. The results do show, however, that isolated transparent layers can fix small amounts of CO_2—presumably a result of PEPC activity.

In all cases, removal of the transparent layer resulted in a significant increase in CO_2 fixation. Thus, although the transparent layer epidermis has been shown to have a few stomata on the ventral side among the stylar hairs (4) the results suggest that transport through these and/or through the pericarp epidermis could limit CO_2 uptake. An examination of the effect of light/dark sequences on stomatal behavior using thin strips of pericarp epidermis from the brush end of the ventral side of a barley caryopsis suggested that up to half of the stomata may be unresponsive to environmental changes (LM Craig, unpublished data). Thus, it seems likely that CO_2 entering the caryopsis may do so via the cuticle of the pericarp epidermis. Removal of the transparent layer then exposes the chloroplast-containing cross-cells to higher concentrations of externally supplied CO_2 and also to higher photon flux density. This would then account for the observed increases in rates of CO_2 fixation.

The logarithmic increases in rates of CO_2 fixation with time of incubation may be a consequence of photosynthetic induction phenomena. In the CO_2 fixation experiments, caryopses were not preilluminated. Under these circumstances, a lag or induction period from a few to 10 or more min is commonly observed (9). This may be due to delayed stomatal opening or to the requirement for autocatalytic buildup of intermediates of the photosynthetic carbon reduction cycle.

The rates of CO_2 fixation in the dark are low, and presumably a result of PEPC activity. The increase caused by removal of the transparent layer with its restrictive effects on CO_2 uptake is no doubt a function of increased CO_2 concentration in the region of the cross-cells. From the data in Table I, in which a total of around 1126 pmol C are recovered in the barley caryopsis over a 15 min incubation in the light, it can be calculated that this is sufficient to account for only about 2% of the weight of starch (approximately 25 mg) found in the mature caryopsis. Assumptions made include: (a) maximum rates of CO_2 fixation are maintained for 18 h d^{-1} over a 20 d grain-filling period; (b) all CO_2 fixed remains in the caryopsis. It is not possible to compare these data with those reported by Evans and Rawson (10) as figures for individual caryopses were not reported. It may be then that the major function of the pericarp is in the refixation of internally derived CO_2 rather than in the fixation of atmospheric CO_2.

CO_2 fixation in the Chl-less mutant was shown to be significantly greater in the light than in the dark. In the presence of the transparent layer, this could be attributed to an increased uptake of CO_2 as a result of light-dependent stomatal opening (but see above). However, as more ^{14}C was recovered in the light than in the dark from *A. lemma* caryopses minus the transparent layer, this cannot be the full explanation. In any case, light-dependent stomatal opening must depend on the presence of chloroplasts in the guard cells. It is not known if the pericarp stomata are functional and whether or not there are chloroplasts in the guard cells. CO_2 fixation in the mutant is presumably catalyzed by PEPC since no Rubisco activity could be detected in the cross-cells and, although not determined in the present work, Rubisco activity has never been detected in transparent layers of a number of barley and wheat cultivars. Clearly, the fixation of CO_2 in the mutant is light dependent. The mechanism from this is unknown. It is noteworthy that the albino mutant assimilates 14 times more CO_2 in the dark than does cv Midas.

The source of energy required to drive the biosynthetic reactions associated with CO_2 fixation is unknown. Electronmicrographs of the cross-cells from *A. lemma* (Dr. M. P. Cochrane, personal communication) show that they contain plastid-like organelles in which starch granules are present. These plastids are not amyloplasts, but it seems likely that they are supplied with starch percursor molecules from elsewhere in the grain that may then be incorporated into insoluble starch in the form of granules. It may be, therefore, that the starch of these plastids is the source of energy for CO_2 fixation in the Chl-less pericarp cells.

The results show that intact caryopses have the ability to translocate assimilates derived from fixation of atmospheric CO_2 to the endosperm/embryo. In general, the amounts are low with by far the greater incorporation being into soluble rather than insoluble material. Translocation of fixed CO_2 is greatest in the albino mutant where, after a 10 min incubation, 30% of the total CO_2 fixed was recovered in the endosperm.

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