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Oxygen Exchange in the Pericarp Green Layer of Immature Cereal Grains[1]

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ANTHONY R. NUTBEAM[2] AND CAROL M. DUFFUS
Department of Agricultural Biochemistry, School of Agriculture, University of Edinburgh, West Mains Road, Edinburgh EH9 3JG Scotland

ABSTRACT

Rates of oxygen exchange in light and dark were recorded for immature detached barley spikelets and wheat florets both before and after successive removal of the husk (palea and lemma), transparent layer of the pericarp, and green layer of the pericarp. Results were compared with those for the mutant barley *Albino lemma* which has a pericarp lacking chlorophyll. There was no net oxygen evolution in the intact spikelets of *Albino lemma* when incubated in the light. Removal of the husk increased the rate of measured oxygen uptake in both light and dark. With normal barley and wheat, net oxygen evolution in the light was observed in intact spikelets and florets, as well as after husk removal and after both husk and transparent layer removal. Additional removal of the green layer of the pericarp resulted in a dramatic changeover from oxygen evolution in the light to oxygen uptake. The results suggest that some of the oxygen generated by pericarp photosynthesis remains within the grain.

The dry matter entering the barley grain is primarily derived from photosynthesis occurring after ear emergence (1), the ear itself contributing up to 76% of the total CO_2 fixed (4). In wheat all of the separated parts of the immature ear, including outer glumes, lemmas, paleae, rachis, and grains, are capable of light-dependent CO_2 fixation (3). The separated grain can account for about 40% of gross photosynthesis and this is presumably derived from CO_2 fixation in the Chl-containing layers of the pericarp. During most of the grain-filling period the pericarp is a bright emerald green tissue surrounded by a transparent outer layer. Previous work (2, 5) with barley has shown that the green layer is capable of photosynthesis and that a number of enzymes normally associated with photosynthetic CO_2 fixation are present. These include ribulose bisP carboxylase and P-enolpyruvate carboxylase. Thus, although the pericarp is capable of CO_2 fixation the significance of its location within the tissues of the ear is not clear.

The aim of the present work was to investigate O_2 exchange in the green layer of the pericarp in immature cereal grains by comparing results obtained in barley spikelets and wheat florets with those obtained under the same conditions for a mutant variety of barley, *Albino lemma*, which lacks Chl in the pericarp. O_2 exchange measurements were selected as a convenient measure of photosynthesis, which in the dark, would additionally give an indication of respiratory activity.

MATERIALS AND METHODS

Plant Material. The barleys, *Hordeum distichum* (L.) Lam. var. Julia and *Hordeum vulgare* (L.) var. *Albino lemma* and the wheat, *Triticum aestivum* (L.) var. Maris Dove, were grown in greenhouses with natural daylength extended to 18 hr with mercury vapor lamps. All plants were grown in soil at a density of six plants/pot (18-cm diameter).

Measurement of O_2 Exchange. Three spikelets or florets were placed in the chamber of a Rank oxygen electrode with 3.0 ml of a solution containing 50 mM Tricine-KOH (pH 7.5), 1 mM $MgCl_2$, 1 mM $MnCl_2$, and 330 mM sorbitol, and agitated at constant speed using a magnetic stirrer. Samples were selected from halfway up the ear where growth conditions are similar and variation in weight minimal. The chamber was jacketed by a circulating water supply at 30 C. Initial rates of O_2 exchange were measured in the dark using an aluminum foil shade, when the system had reached steady-state conditions. Afterwards the shade was removed and the chamber illuminated with a 275-w (pearl) Phillips No. 1 Photoflood tungsten lamp, the bulb of which was 15 cm from the center of the chamber. Illuminance within the chamber was 658 μE m^{-2} sec^{-1} (400–700 nm).

The rates of O_2 uptake in the dark and in the light were assumed to be the same. This is certainly true for the transparent layer and the grain when stripped of all photosynthetic tissue. On the other hand, the presence of photorespiration in green tissue would result in an increased O_2 uptake in the light compared to that in the dark. It may be, however, that photorespiration is low in the green layer of the pericarp since ribulose bisP carboxylase activity is low compared to that of P-enolpyruvate carboxylase (2). Glycolate oxidase activity, an additional marker of photorespiration, is also low compared to that found in barley and wheat leaves (A. R. Nutbeam, in preparation).

Each experiment with three grains was conducted three times and the mean of the three rates calculated. The rate of O_2 exchange was expressed in nmol of O_2/min·grain or grain part. Rates of O_2 exchange were also recorded for wheat florets and barley spikelets after successive removal of: (*a*) the husk (palea and lemma); (*b*) the transparent layer of the pericarp; and (*c*) the green layer of the pericarp. As *Albino lemma* pericarp has no green layer the final stage of layer removal was necessarily omitted.

Light-dependent O_2 evolution was also measured in isolated husks and green layers of pericarp.

RESULTS

Net O_2 evolution in the light was observed in intact wheat florets and after successive removal of the husk and transparent layer of pericarp (Table I). Only when the pericarp green layer was removed was there a dramatic changeover from O_2 evolution to O_2 uptake. This figure (-11 ± 1.0 nmol/min·grain) was not significantly different from that (-13.3 ± 1.0 nmol/min·grain), observed in the dark. Removal of the husk caused an increase in light-dependent O_2 evolution from 10 to 18 nmol of O_2/min·grain. The isolated husk had a small but significant rate of O_2 evolution in the light (2.0 nmol/min·grain) which, when corrected for O_2 uptake in the dark, resulted in 6 nmol/min·grain for light-de-

[1] This work was supported by the Agricultural Research Council.
[2] Present address: Department of Biophysics, Chelsea College, 17A Onslow Gardens, London SW7 3AL, England.

pendent O_2 evolution. Removal of the transparent layer of cells covering the pericarp green layer did not significantly change the rates of either O_2 evolution in the light or light-dependent O_2 evolution. However, it did result in a large increase in O_2 uptake in the dark.

Corresponding data for barley are shown in Table II. Spikelets (minus awns), spikelets with the husk removed, and with the husk and the pericarp transparent layer removed showed net O_2 evolution in the light. Successive removal of the husk and the transparent layer resulted in a sharp increase in O_2 uptake in the dark. O_2 evolution in the light was not significantly affected. Removal of the pericarp green layer resulted in a decrease in O_2 uptake in the dark and a dramatic changeover from O_2 evolution to O_2 uptake, in the light. In these experiments O_2 evolution in the light was not observed with isolated husks or pericarps. Taking into account O_2 uptake in the dark, both tissues were capable of light-dependent O_2 evolution.

Data for the barley *Albino lemma* are shown in Table III. No net O_2 evolution in the light was obtained in any grain or grain fraction examined. Successive removal of the husk and transparent layer markedly increased the rate of O_2 uptake in both light and dark. Both intact grains and isolated husks were capable of small but significant rates of light-dependent O_2 evolution. Very low values for light-dependent O_2 evolution in the grains with husk removed and with both husk and transparent layer removed were observed.

Table I. Oxygen exchange in wheat var. Maris Dove

Wheat florets were removed at the stage of development most closely corresponding to that of the barley spikelet at 25 days after anthesis. The tissue used for analysis is that remaining after successive removal of the outer layers described below. Data are the mean of three estimates ± standard deviation and are expressed in nmol O_2/min/floret or tissue fraction.
+.... O_2 evolution; -.... O_2 uptake

Tissue	Oxygen exchange		
	Light	Dark	Light dependent O_2 evolution
Floret	+ 3.0 ± 0.3	- 7.0 ± 0.0	+ 10.0 ± 0.3
Palea and lemma removed	+ 9.0 ± 2.0	- 9.0 ± 0.3	+ 18.0 ± 1.0
Transparent layer of pericarp removed	+ 5.0 ± 3.0	- 16.0 ± 2.0	+ 21.0 ± 4.0
Green layer of pericarp removed	- 11.0 ± 1.0	- 13.3 ± 1.0	+ 3.0 ± 2.0
Isolated husk	+ 2.0 ± 1.0	- 4.0 ± 0.3	+ 6.3 ± 1.0

Table II. Oxygen exchange in barley var. Julia

Spikelets were removed at 25 days after anthesis. The total period between anthesis and full maturity was 60 days. Awns were removed as these were too long to fit in the chamber of the oxygen electrode. The tissue used for analysis is that remaining after successive removal of the outer layers described below. Data are the mean of three estimates ± standard deviation and are expressed in nmol O_2/min/spikelet or tissue fraction. +.... O_2 evolution; -.... O_2 uptake.

Tissue	Oxygen exchange		
	Light	Dark	Light dependent O_2 evolution
Spikelet	+ 5.4 ± 1.6	- 3.8 ± 0.8	+ 9.4 ± 1.1
Husk removed	+ 4.3 ± 2.9	- 8.2 ± 0.6	+ 12.5 ± 3.4
Transparent layer of pericarp removed	+ 1.2 ± 3.5	- 13.3 ± 0.6	+ 17.3 ± 1.7
Green layer of pericarp removed	- 8.4 ± 0.2	- 10.3 ± 0.5	+ 1.8 ± 0.2
Isolated husk	- 1.0 ± 0.4	- 6.2 ± 1.4	+ 5.3 ± 1.8
Isolated green layer of pericarp	- 2.3 ± 0.3	- 2.8 ± 0.2	+ 0.5 ± 0.0

Table III. Oxygen exchange in barley (*Albino lemma*)

Conditions are as described in Table II. Data are the mean of three estimates ± standard deviation and are expressed in nmol O_2/min/spikelet or tissue fraction.
+.... O_2 evolution; -.... O_2 uptake

Tissue	Oxygen exchange		
	Light	Dark	Light dependent O_2 exchange
Spikelet	- 0.9 ± 0.4	- 3.4 ± 0.8	+ 2.9 ± 0.3
Husk removed	- 6.3 ± 0.6	- 7.2 ± 0.3	+ 1.0 ± 0.6
Transparent layer of pericarp removed	- 6.6 ± 0.3	- 8.4 ± 0.4	+ 1.8 ± 0.6
Isolated husk	- 1.1 ± 0.1	- 3.4 ± 0.3	+ 2.3 ± 0.3

DISCUSSION

In *Albino lemma* both in light and dark and in Julia in the dark, removal of the palea and lemma resulted in a marked increase in O_2 uptake. This suggests that these outer layers may limit the influx of atmospheric O_2. In wheat the results were less marked and in the dark the slight increase in O_2 uptake resulting from the removal of the husk indicated that in this case the palea and lemma may have been less of a barrier to O_2 influx than in barley. The increase in light-dependent O_2 evolution upon removal of the palea and lemma, which was greater in wheat than in barley, suggests either that these tissue constitute a barrier to O_2 efflux from the green layer of pericarp or that the increased incident light resulted in higher rates of photosynthesis and hence of O_2 evolution or that both factors contributed to the effect. Certainly, if the outer layers were entirely permeable, the respiration rate would be expected to fall on their removal as these tissues would no longer be present to add their O_2 consumption to the total. In the case of *Albino lemma* the rates of O_2 uptake in intact grains were similar to those in the isolated palea and lemma, suggesting that rates of O_2 evolution measured for intact grains might be entirely accounted for by these tissues.This was not so clear cut in Julia or Maris Dove. If O_2 access to the grain is restricted by the outer layers then the measured rates of respiration may not reflect *in vivo* rates since O_2 levels may be limiting. In addition, if CO_2 efflux is simultaneously prevented then, in the absence of internal refixation, its concentration will rise until respiration is reduced by lack of O_2.

Since removal of the transparent layer of the pericarp increased the rates of respiration markedly in both wheat and Julia and rather less in *Albino lemma* it is likely that this tissue may also be a barrier to O_2 uptake. At the same time, rates of light-dependent O_2 evolution increased. Thus, this layer may have properties similar to those described above for the palea and lemma.

Removal of the green layer of pericarp in wheat and barley resulted in similar rates of O_2 uptake in both light and dark. It can therefore be concluded that this layer is responsible for the observed rate of light-dependent O_2 evolution by isolated dehusked spikelets or florets. Since the green layer of pericarp dehydrates extremely rapidly on removal from the grain it was perhaps not surprising to observe no net O_2 evolution in the light using isolated pericarps. The observed O_2 uptake was presumably due to active respiration in the presence of nonlimiting supplies of O_2. While some damage inevitably occurred during spikelet and floret removal, little or no apparent dehydration of endosperm or outer layers was observed during the course of the experiment.

The results suggest that some of the O_2 generated by pericarp photosynthesis remains within the grain. The source of O_2 for endosperm respiratory processes may thus be the pericarp rather than the outer atmosphere.

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