Starch synthesis in developing wheat grain

The effect of light on endosperm starch synthesis in vitro and in vivo

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Abstract. The effect of light on the in-vivo rate of starch synthesis in the endosperm of developing wheat (*Triticum aestivum* cv. Mardler) grain was studied. Individual grains from spikelets grown on the same spike either in darkness or bright light showed no difference in their ability to accumulate radioactivity or to convert this to starch over a 14-h period. Similarly, there was no difference in final grain dry weight between spikes which had been kept in either darkness or normal light from 10 d post anthesis. In contrast, when “half-grains” (grain which had been bisected longitudinally along the crease region) were incubated by being submerged in culture solution (in vitro) the incorporation of [14C]sucrose into starch was stimulated by increased irradiance. Further experiments showed that the in-vitro dependence on light could be linked to the availability of oxygen. We suggest that in vitro the diffusion of oxygen into the endosperm cells combined with an increased rate of respiration of the tissue during the incubation causes this limitation. Thus the dependence of starch synthesis on light is an artefact of the in-vitro incubation system. The photosynthetic ability of the green pericarp tissue can be used to prevent the development of anoxia in the endosperm tissue of half-grains incubated in vitro. In conclusion, we propose that starch synthesis in vivo is not dependent on oxygen production by photosynthesis in the green layer of the pericarp.

Key words: Endosperm – Grain filling – Pericarp – Photosynthesis – Starch synthesis – *Triticum* (starch synthesis)

Introduction

The developing wheat grain contains an inner starch-storing endosperm surrounded by an outer chlorophyll-containing layer of pericarp tissue. During most of the grain-filling period the pericarp tissue is green and capable of producing oxygen by photosynthesis (Nutbeam and Duffus 1978). Furthermore, because the outer pericarp tissue is relatively impermeable to carbon dioxide (Radley 1976) and to oxygen (Nutbeam and Duffus 1978) it has been suggested that a major function of the green layer may be the provision of oxygen to maintain starch synthesis in the inner endosperm tissue (Duffus 1979; Cochrane and Duffus 1979). Thus it was postulated than the rate of starch deposition in cereal endosperm is regulated, in part, by oxygen derived from green-layer photosynthesis.

This attractive hypothesis received some support following the observation that [14C]sucrose incorporation into starch by grain incubated in vitro is sustained by the photosynthetic production of oxygen in the green layer of the pericarp (Gifford and Bremner 1981). These authors suggested that this light-oxygen effect provides a plausible explanation for the shell structure of wheat starch granules, which appears to correlate with the number of light-dark cycles during grain development (Buttrose 1962).

We have examined the effect of light on starch synthesis in developing wheat grain under both in-vivo and in-vitro incubation conditions. Our aim was to establish whether or not starch synthesis in vivo was limited by the availability of oxygen derived from pericarp photosynthesis.

Material and methods

Plant material. A continuous supply of developing wheat (*Triticum aestivum* cv. Mardler, National Seed Development Organisation, Newton, Cambridge, UK) grain was achieved by the following procedure: approx. 40 seeds were grown each week in John Innes No. 3 compost. After one week in a greenhouse the seedlings were moved to a cold room for vernalisation for six weeks at 4°C with light from fluorescent lamps for 8 h per day. The vernalised seedlings were transplanted, two per pot, into 15-cm plant pots and returned to the greenhouse. Supplementary lighting was supplied from September until April for 16 h per day using sodium-vapor...
lamps; giving photosynthetically active radiation of approx. 500 μmol photons m\(^{-2}\) s\(^{-1}\) (measured using a Crump quantum photometer; Crump Instruments, Billericay, Essex, UK). Fungal disease was controlled with Milgo fungicide (ICI, Furnhurst, Surrey, UK) and insect pests were controlled with Fumite DDT/Lindane insecticide generators (Pains-Wessex, Salisbury, Wiltshire, UK). After the emergence of the third leaf, the plants were fed each week with 0.1 g per pot Phostrogen fertiliser (Phostrogen, Corwen, Clwyd, UK). The developing ears were tagged with the date of anthesis.

In-vivo experiments. Greenhouse-grown plants were transferred at 20 d post anthesis (dpa) to a growth cabinet (Fisons, Loughborough, UK) in continuous light (both tungsten and fluorescent lamps) at a photon fluence rate of 400 μmol m\(^{-2}\) s\(^{-1}\) at 20° C and 80% relative humidity. Using an adaptation of the technique described by Kolderup (1979), radioactively labelled sucrose (2960 kBq [U-\(^{14}\)C] sucrose in 20 mM sucrose) was supplied to the plant. The labelled sucrose was placed in a reservoir attached to the stem and a small nick was made in the stem to allow uptake of the solution from the reservoir.

Chemicals. [U-\(^{14}\)C]Sucrose (≥ 13 GBq mmol\(^{-1}\), 350 mCi mmol\(^{-1}\)) was purchased from Amersham International PLC (Amersham, Bucks, UK). All other chemicals were obtained from either Sigma Chemical Co. (Poole, Dorset, UK) or BDH Chemicals (Poole, Dorset, UK).

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In one experiment, plants were grown with ears covered with either cellophane bags or the same bags covered with black paper as described below in “Analysis of radioactivity”.

In one experiment, plants were grown with ears covered with either cellophane bags or the same bags covered with black paper to exclude light. This treatment was imposed at 14 dpa to exclude effects on the cell-division phase of grain development. At grain maturity (55 dpa), grains from the central region of the spike were dried and weighed.

In-vitro incubation conditions. At 21 dpa, pairs of grain were removed from the basal florets of the spikelets in the central region of the spike and weighed. Grains were then bisected longitudinally along the crease region and were quickly transferred to 25-ml conical respirometer flasks with 3 ml of incubation medium that contained 10 mM 2-(N-morpholino)ethanesulphonic acid (Mes) pH 6.5, 60 mM KCl and a range of sucrose concentrations varying from 10 mM to 100 mM plus 37 kBq [\(^{14}\)C]sucrose unless stated otherwise. In most experiments, the flasks were illuminated with two 300-W tungsten-halide lamps giving a photon fluence rate of 500 μmol m\(^{-2}\) s\(^{-1}\) (Crump quantum photometer). A 3-cm-deep tray of water was placed between the light source and the flasks to prevent overheating. Different irradiances were obtained by varying the distance between the lamps and the flasks. Dark treatments were provided by covering the flasks with black tape. At the end of the incubation period the half-grains were removed from the incubation medium and briefly rinsed three times with 15 ml distilled water. The endosperm tissue was quickly excised and treated as described below in “Analysis of radioactivity”.

Analysis of radioactivity from in-vivo and in-vitro treatments. Endosperm tissue from grain incubated in vivo or in vitro was homogenised in 2 ml of ice-cold 1 M perchloric acid (PCA) using a Polytron vortex homogeniser. The homogenisation probe was then washed with a further 1 ml of 1 M PCA, which was combined with the original PCA-homogenate. The tissue homogenates were centrifuged at 4000 g for 10 min at 4° C, and the supernatant (PCA-soluble fraction) was decanted. The starchy pellet (PCA-insoluble fraction) was washed three times by resuspending in 3 ml of ice-cold distilled water and centrifuging as above. The supernatant of the first wash was combined with the PCA-soluble fraction and subsequent washes discarded.

The PCA-soluble fraction was adjusted to pH 6.5-7.5 using 5 M KOH and 1 M KH\(_2\)PO\(_4\). The insoluble potassium-perchlorate precipitate was removed by centrifugation at 4000 g for 10 min at 4° C. The PCA-soluble fractions were kept at 4° C during processing.

The washed PCA-insoluble fraction was resuspended in 5 ml of 0.1 M sodium-acetate buffer pH 4.5, and boiled for 30 min with periodic shaking to resuspend the gelatinised starch. After cooling, the gelatinised starch was degraded enzymatically to glucose by incubation overnight at 37° C with 56 units of amyloglucosidase (EC 3.2.1.3; Sigma grade V). Any insoluble material remaining was removed by centrifugation at 4000 g for 15 min. Glucose in the supernatant fraction was measured using a Boehringer Kit (number 124010; Boehringer, Mannheim, FRG) which is based on the glucose-oxidase method described by Werner et al. (1970). Radioactivity in the supernatant of the enzyme-digested PCA-insoluble fraction was measured by liquid scintillation counting, after mixing 1 ml of the samples with 10 ml of lumagel scintillant (LKB Wallace Inc, Hinton, USA). Counting efficiency was determined in each sample by the sample channels ratio method.

Measurement of grain oxygen exchange. At 21 dpa, pairs of grain were removed from the basal florets of the spikelets in the central region of the spike. Ten half-grains were prepared and incubated as described above under ‘in-vitro incubation conditions’, in 3 ml of incubation medium containing 100 mM sucrose. At various times during the incubation, the half-grains were briefly transferred to fresh medium in a Rank oxygen electrode (Rank Instruments, Boffstham, Cambridge, UK) to measure their oxygen consumption in darkness and oxygen evolution in bright light (500 μmol m\(^{-2}\) s\(^{-1}\)).