Chloroplast development in low light-grown barley seedlings

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Abstract. Segments of 7-d low light-grown barley laminae cut at 0.5 cm intervals up from the intercalary meristem were examined ultrastructurally and biochemically. The different regions upwards showed the succession of plastid development in light-grown tissues of eoplasts, amyloplasts, amoeboid, immature and mature plastids as described by Whatley (1977). Semi-crystalline bodies were detected in all of them. The eoplast-amyloplast regions are characterised by a greater proportion of mitochondria and high levels of ATP and 3-phosphoglyceric acid, together with low levels of inorganic phosphate conducive to the activation of ADP glucose pyrophosphorylase. The amoeboid and immature plastid regions have higher levels of inhibitory phosphate and starch breakdown may be responsible for the release of metabolites and energy for development. Segments containing amoeboid and immature plastids also have reduced levels of ATP (and 3-phosphoglyceric acid) as photosynthetic components are synthesised. Using ultrastructural assessments of areas of thylakoids, first β-carotene and violaxanthin, followed by chlorophyll a and lutein and, lastly, chlorophyll b are concentrated in the developing lamellar systems of the immature and mature chloroplasts. The formation of additional membraneous material which spreads these pigment systems over a greater thylakoid area within the plastids is the final stage of plastid morphogenesis in low light-grown seedlings.

Key words: Amyloplast – Chloroplast development – Hordeum – Starch.

Introduction

In studies of higher plant chloroplast development, well over 95% of literature in the past refers to the ‘greening’ of etiolated tissue involving the conversion of etioplasts to mature plastids. There have been many reasons for this but principally this system enabled a ‘slowing-down’ of parts of the developmental process so that they could be studied in detail. As a result much is now known about the sequential assembly of the photosynthetic apparatus. Furthermore, the rôle of light quality particularly at the red end of the spectrum in controlling many aspects of development could only have been evaluated by experimenting with dark-grown tissue (e.g. Kasemir 1979). Nevertheless, with the advent of more sensitive techniques, biochemical and physiological studies of plastid development in light-grown tissue grown under more normal conditions and similar to those in the field are possible provided that a sound and comprehensive understanding of the basic ultrastructure of the system underpins each study.

Ultrastructural assessments have been made of dicotyledonous and mono-cotyledonous light-grown plants. Whatley (1974, 1977) has shown that the plastids in all cells of the primary leaves of light-grown Phaseolus vulgaris undergo the same sequence of structural changes and has identified 7 basic and distinct stages with optional diversions in several species. The first three proplastid stages she describes, namely those of the eoplast, amyloplast and amoeboid plastids, are quite unlike plastids in mature etiolated or greening tissue.

Monocotyledons differ developmentally from dicotyledonous plants by virtue of having a basal intercalary meristem (Esau 1953). Leech and co-workers have undertaken a series of light-grown studies using maize (Zea mays) and lately wheat (Triticum aestivum). Leese et al. (1971) first showed that different populations of plastids could be effectively prepared from segments taken along the length of the maize seedling which correspond with the in situ appearance of the plastids. Using this system, Leech and co-
workers have examined lipid biosynthesis (Hawke et al. 1974), photochemical activities (Baker and Leech 1977) and changes in pool sizes of amino acids and amides (Chapman and Leech 1979). Previously Robertson and Laetsch (1974) had shown that the age of developing etiolated tissue has a considerable effect on the rate of chlorophyll formation during greening. Boffey et al. (1980), using light-grown wheat to avoid dimorphism, have extended this to show that the greening of mature etioplasts cannot be used as a model for the normal development of proplastids into mature chloroplasts, as not only exposure to light, but also chronological age of the plastid are important factors in development.

In the past we have endeavoured to examine the sources of energy for the development of plastids whilst effective photophosphorylation is absent and have shown that mitochondria are specifically synchronized within the greening process (Hampp and Wellburn 1980). Consequently it is of interest to know if this is also a feature of plastid development in light-grown tissues. This paper describes a series of experiments which, whilst not demonstrating mito-

chondrial-plastid co-operation, reveal several features of light-grown plastid development that are not associated with etioplast-chloroplast transformations. The lower levels of light and temperature, by comparison to other studies, employed in this study were deliberately chosen not only to slow down the early stages of light-grown development but to simulate as close as possible the cultivation conditions around soil level for autumn-sown cereals in latitudes about 53° N.

Material and methods

Pre-soaked (8 h) seeds of barley (Hordeum vulgare L. cv. Ark Royal) were germinated overnight on the surface of moist peat, lightly dusted the following morning with peat and then grown for the next six-full days in a light/dark (16 h/8 h) regime at 20 °C on a moist capillary bed. The light fluence rate was determined by the appropriate probes of a Li-Cor Quantum Photometer (Model LI-185) to be 5 μmol m⁻² s⁻¹ (250 lux). Under these conditions, at 7 days from soaking, the lengths of the laminae were 8 to 9 cm and the coleoptiles approximately 2.5 cm. By exerting a gentle but steady pull on the blades they usually break below the intercalary meristem and the laminae slide out from the coleoptiles revealing white tissue immediately above the node of the meristem about 1 cm in length with a short distinct yellow to yellow-green region before merging into the full green of the remainder. After practice several hundred laminae can be harvested within a short period, placed with their meristems in line on a glass thin layer chromatography plate (with squared graph paper beneath) and the lengths of the arrayed laminae severed with a fresh and degreased razor blade at 0.5 cm intervals. These different populations of segments were used for a variety of determinations. Usually one hundred sections were sufficient but occasionally 500 were required if sensitivity became a limitation. For electron microscopy 1 mm sections taken just above the meristem and from the middle of the different 0.5 cm sections were processed according to a procedure described earlier (Wellburn and Wellburn 1979). Areas and distances on electron micrographs were measured using a Graphics Tablet attached to an Apple mini-computer. For each segment between 50 and 100 micrographs were employed. The mean values of the top 10% of the size distribution range of organelles and starch grains were taken as those most likely to represent the centres of the structures and are referred to as maximum areas. It was using such plastids that the lengths of the internal membrane (L) were measured. The total thylakoid area was calculated per plastid, per cell and per 0.5 cm segment by determining a notional radius from the circumference (L)/2π and finding the value for 4πr². In addition longitudinal and transverse sections of the 0.5 cm segments from each region were examined by light microscopy. The number of cells in each dimension were counted and hence the total number per tissue volume calculated. 

Protoplasts were made from 100 of each of the sections by the procedure of Hampp and Ziegler (1980) and examined using a phase-contrast fluorescence microscope. By moving through the depth of field individual plastids in the protoplasts were counted and the number of plastids per cell for the different regions of the laminae obtained.

Levels of total ATP in extracts from the different segments were measured by bioluminescence using the method described by Wellburn et al. (1981) and energy charge ratios were calculated by the procedure adopted by Hampp and Wellburn (1980). Phos-

phate was determined by the method of Taussky and Shorr (1953) and total protein by that of Lowry et al. (1951). Amounts of phospho-glycerate in different segments were estimated in triethan-

olamine buffer (0.1 M, pH 7.6) using ATP in the presence of 3-

phosphoglycerate kinase to form ADP and 1,3-diphosphoglycerate and following the disappearance of ATP by bioluminescence. Crude extracts from the different segments were prepared using the method of Ozbum et al. (1972) and α-amylase and β-amylase activities were determined by the method of Abbott and Matheson (1972). Starch synthetase was estimated in similar extracts by add-

ing pyruvate kinase and phospho-enol pyruvate to convert ADP from ADP-glucose to ATP which was measured by bioluminescence.

Pigments were rapidly isolated from segments by maceration in 50% (v/v) methanol for 15 seconds using an Ultraturrax with a miniature probe, adding triple the volume of chloroform and shaking vigorously. The phases were separated using centrifugation (100 g for 3 min) and the lower chloroform layer, containing virtually all the colour, was evaporated under O₂-free N₂. Half was redissolved in high performance liquid chromatography (HPLC) eluting solvent (methanol:acetone:water, 67.5:25:7.5% by vol.) and the remainder in di-ethyl ether for determination of total chloro-

phylls and carotenoids using the method of Gaudillere (1974). Pigments were separated isocratically by reverse-phase (C18) HPLC (Spectra-Physica Model 3510) through a 250 mm column (4.6 mm internal diameter) packed with Partisil 10 ODS (Whatman 10 g) using an Ultraturrax with a miniature probe, adding triple the volume of chloroform and shaking vigorously. The phases were separated using centrifugation (100 g for 3 min) and the lower chloroform layer, containing virtually all the colour, was evaporated under O₂-free N₂. Half was redissolved in high performance liquid chromatography (HPLC) eluting solvent (methanol:acetone:water, 67.5:25:7.5% by vol.) and the remainder in di-ethyl ether for determination of total chloro-

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Pigments, metabolites and levels of enzymic activity were determined in triplicate from three separate experiments each. Where appropriate the error bars on the different figures represent the standard deviations of each set of determinations. Graphs have used 'length up laminae' as the developmental parameter; representing that distance as measured from the node of the intercalary meristem upwards towards the tip of the monocotyledonous blade. The levels of components are expressed in terms of protein content rather than per segment. Of major interest in this study were the