Influence of Cell Age on Chlorophyll Formation in Light-grown and Etiolated Wheat Seedlings

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ABSTRACT

A method is described for relating the age of a cereal leaf cell to its distance from the leaf base. The rates of chlorophyll synthesis per plastid in the first leaf of light-grown and of greening etiolated seedlings of wheat (Triticum aestivum, var. Maris Dove) increase with cell age. Normally developing plastids of light-grown wheat take over 24 hours to reach the chlorophyll a/b ratio characteristic of mature wheat chloroplasts (4.5), but mature etioplasts need only 6 hours light to achieve this a/b ratio. Plastid greening potential depends only on cell age, whereas the chlorophyll a/b ratio is influenced both by cell age and by light.

The seedlings of many cereals are particularly well suited to the study of chloroplast development, since the developing plastids are in a linear series, with the youngest in cells near the base and the oldest in cells near the tip of the leaf. Robertson and Laetsch (14) took advantage of this arrangement to study Chl synthesis during the greening of etiolated maize seedlings, and demonstrated that the age of developing etioplastic tissue had a considerable effect on the rates of Chl synthesis following illumination. They reported that the rate of greening, per g fresh weight, was greater in regions distant from the leaf base than in the younger regions near the base. Mackender (11) found a similar increase in greening potential with cell age when he measured the levels of protochlorophyllide/g fresh weight in sections cut at various distances from the bases of etiolated barley leaves.

A greening etiolated leaf is far from being a natural phenomenon, and it is important to establish whether cell and plastid age also influence the kinetics of Chl biosynthesis in chloroplasts of seedlings grown under a normal diurnal light regime. One indication that significant differences may exist, at least in some species, between plants subjected to the two different regimes, is the finding that the Chl a/b ratio is constant even in the youngest plastids of a young, green maize leaf (3); this is in contrast to the rapidly decreasing Chl a/b ratio characteristic of mature, greening etiolated leaves.

A close comparison between greening etiolated and light-grown plants of the same species would help to pinpoint the similarities and differences between the two systems. Any such comparison should be in terms of rates of synthesis and levels of Chl/plastid, and should ensure that cells of the same age are being compared.

This paper presents the results of a study of this kind. We have examined plastid development in wheat in order to avoid dealing with dimorphic chloroplasts, and because we already have a good background knowledge of the development of wheat seedlings (5). Methods are described for measuring the leaf tip growth rate over several days before harvest, and the growth rate at any position in the leaf relative to the growth rate at its tip, for both etiolated and light-grown wheat seedlings. Using these measurements it has been possible to convert distance from the leaf base into cell age, and so to make direct comparisons between chloroplasts and etioplasts in cells of the same age in light-grown and greening etiolated seedlings.

MATERIALS AND METHODS

Plant Material. Seeds of wheat, Triticum aestivum, var. Maris Dove (Dickson, Brown & Tait Ltd., Altrincham, U.K.) were soaked in running tap water at 20°C and surface-sterilized in sodium hypochlorite solution (13% free chlorine) after 1 h. After being soaked for an additional 16 h, the seeds were sown in Levington Universal Compost (Fisons, U.K.) at a depth of 1 cm. Light-grown seedlings were given a photoperiod of 16 h at 20°C with a 5°C night temperature and 70% RH. The light intensity at the level of the seedlings, measured with a solarimeter (Kipp & Zonen), was 4.0 mw.cm⁻². Dark-grown seedlings were grown in total darkness at 25°C, about 100% RH. After 5.5 days from sowing dark-grown seedlings were allowed to green in the light (intensity 1.3 mw.cm⁻²) at 25°C and about 100% RH. These conditions were chosen because they had been shown to be suitable for the rapid development of photosynthesis in greening barley (7).

Measurement of Growth Rates. To measure total leaf growth rates, 20 randomly chosen seedlings were labeled individually, 4 days after sowing, and the heights of their tips above soil level were measured at intervals over the next 4 days. The rates of growth at intervals of 1 day were calculated for each seedling, averaged for the entire sample of 20, and plotted against age of leaf.

To measure the growth rates of different regions within the same leaf, the first leaves of seedlings were marked at 0.5 cm intervals from the base to the tip with ink spots, removing a strip of coleoptile in order to mark the leaf where necessary. The heights of these spots above soil level were measured periodically, and the rates of their upward movement were expressed as a fraction of the rate of growth at the leaf tip. These relative growth rates were plotted against distance from the leaf base, using only seedlings whose tip growth rates were unaffected by the marking process.

Computation of Cell Age. Two features of young monocotyledonous grass leaves have a large influence on their pattern of growth. (a) All cell division occurs in a basal meristem. Cells are formed here by repeated divisions of the meristematic cells, and, for the purposes of this analysis of growth, the age of a cell can be...
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measured from the time it is cut off from the meristem by its final cell division. Cells do not divide again once they have been displaced from the meristem. (b) There is a zone of constant length in the lower part of the leaf, extending from the basal meristem to a fixed height above the leaf base, in which all the cells are elongating. Above this zone of elongation no further increase in cell length occurs.

These features of cell and leaf growth are illustrated diagrammatically in Figure 1, which shows the positions of cells in a monocotyledonous leaf at daily intervals over the 3 days immediately before harvest. Day 4 (3 days before harvest): cell A has just been cut off from the basal meristem. Cell A is small (about 20 μm in length), approximately cube-shaped, and will not divide again; it will, however, elongate. Day 5 (2 days before harvest): cell A has been displaced a short way up the leaf by the cutting off and elongation of newer cells between cells A and B, formed by the basal meristem during the preceding day. Cell A itself has elongated, and cell B has just completed its last division. Day 6 (1 day before harvest): cell A has been pushed much farther away from the leaf base, owing to the combined elongations of all of the cells below cell A, approximately half of which have been produced by the basal meristem over the preceding day. Cell A is now just above the zone of elongation, and has, therefore, reached its maximum length. Cell B has elongated and has been displaced upwards a little more than was cell A during its first day, because the rate of meristematic divisions and/or the rate of cell elongations were greater between days 5 and 6 than between days 4 and 5. This resulted in an increased rate of leaf growth, measured at the leaf tip. It is evident that cells A and B are farther apart by day 6 than they were at day 5. Cell C has just completed its last division. Day 7 (harvest): both cell A and the leaf tip have been displaced upward by the same distance during the previous day, as both were above the zone of elongation, and so did not change their distance apart. Cell A has remained the same size as at day 6, and has been moved a little farther from cell B, owing to the elongation of some cells above B. Similarly, B and C have moved farther apart, and both have elongated. Cell D has just completed its final division.

At harvest, cells A, B, and C are, respectively, 3, 2, and 1 days old, and their positions along the leaf are certainly not directly proportional to their ages. To calculate the position at harvest of a cell of any given age, the rate of upward displacement must be known for any region of the leaf, at any time before harvest up to the age of the cell. The cell position can be calculated from the graph of tip growth rate against time after sowing, and the relative growth rate at any point up the leaf, measured as described above. Several equations are involved. $T = \text{days before harvest}$, $d = \text{distance from leaf base}$. Tip growth rate $= R_T \text{cm/day at time } T$, relative growth rate $= \frac{\Delta h}{\text{cm from leaf base}}$, absolute growth rate $= r_{T,d} = R_T \cdot x_d \text{cm/day}$. Time, $\Delta t$, taken for a cell to be displaced a small distance upwards, $\Delta d$, is given by

\[ \Delta t = \frac{\Delta d}{r_{T,d}} \]

The nature of the functional relation of $r_{T,d}$ to $d$ is unknown, and so values of $x_d$ were read from a graph, as described above, at 1-mm intervals from the leaf base, and stored in a computer. Each graph of tip growth rate against time before harvest was treated as a series of joined straight lines. For light-grown wheat, for example linear interpolation was used between 2.5 days (2.35 cm/day) and 1.5 days (3.5 cm/day) before harvest; the rate remained constant thereafter.

A computer was programmed to use the stored values of $x_d$ and the equation relating to $R_T$ to $T$, to calculate the height at harvest of a cell of any given age, $T$. This was achieved by using the equations above to determine the times taken for a cell of age $T$, to be displaced upwards from the leaf base by successive steps of 0.1 cm.

$t_d$ was first calculated for $d = 0$ and $T = T_n$, then for $d = 0.1$ and $T = (T_n - t_0)$, then for $d = 0.2$ and $T = (T_n - t_0 + t_0)$, etc., until the total time taken equalled $T_n$, when the height reached was equal to the height above the leaf base of a cell, age $T$, days.

This series of calculations was repeated for a sufficient number of cell ages to allow a conversion graph of cell age against distance from the leaf base to be plotted. Almost all cell divisions occur in the bottom 0.5 cm of the leaf (5), so little error is introduced by treating the meristem as if it were infinitely thin and located at the leaf base. The values of $x_d$ and the equations for $R_T$ were different for the light-grown and the etiolated seedlings, and must be newly determined for each variety of plant and set of growth conditions used.

Harvesting. Light-grown seedlings were harvested 7 days after sowing, 2 h after the start of a light period; the leaves were cut at their bases and the coleoptiles gently pulled off. Greening etiolated seedlings were harvested at intervals during greening and their coleoptiles removed. The leaves were cut into sections (0.5 cm if light-grown, 1.0 cm if greening) at various heights above the leaf base, to allow plastids to be isolated from cells of various ages. Cells at a given height above the leaf base at the start of greening were about 1.4 cm higher up the leaf 8 h later, so for greening experiments the positions of sectioning were moved upwards at successive harvests to keep pace with leaf growth.

Plastid Isolation. Plastids were isolated by chopping leaf sections with a razor blade in an isolation medium containing 0.4 M sorbitol, 0.75 mM MgCl₂, 50 mM Heps (pH 7.6). The slurry was filtered through eight layers of nylon bolting cloth (25 μm mesh) and layered onto 1 ml isolation medium containing 0.4 M sucrose instead of sorbitol. After centrifugation for 5 min at 900gmax, the pellet was resuspended in the medium containing sorbitol. All manipulations were performed on ice or under refrigeration at 4°C, in dim natural light.

Chl Assays. To determine their Chl content and Chl a/b ratio, the isolated plastids were counted in a haemocytometer and the Chl measured according to Arnon (1) using a Unicam SP500 spectrophotometer. Arnon's method is not very well suited to the measurement of high (greater than about 6) Chl a/b ratios (13), so the a/b ratios of Chl extracted from sections of leaf at various distances from the base were measured according to Ogawa and Shibata (13), using at least 150 leaves for sections up to 3.0 cm from the base, and 50 leaves for sections higher up the leaf. Optical density measurements were performed using a Unicam SP1800 spectrophotometer, slit width 0.3 mm.

![FIG. 1. Diagram of the leaves of light-grown wheat seedlings 4, 5, 6, and 7 days after sowing, to illustrate relative positions and sizes of cells A, B, C and D, cut off from the basal meristem at intervals of 1 day. The cells are not drawn to the same scale as the leaves, and some features of the leaf growth have been exaggerated in order to illustrate them more clearly. In this example, time of harvesting is assumed to be 7 days after sowing.](www.plantphysiol.org)
RESULTS

Rate of Chl Synthesis. In normally-grown wheat seedlings the amount of Chl/plastid rose almost exponentially with increasing distance from the leaf base to about 5 cm; above 5 cm the increase in rate was less dramatic (Fig. 2). In order to determine rates of Chl synthesis/plastid at different stages in their development, the relationship between the height of the cell above the leaf base and the cell age was first established, as the age of a cell is not directly proportional to its distance from the leaf base. The tip growth rate of light-grown seedlings was found to reach a maximum of 3.5 cm/day, 36 h before harvest, and remained constant up to harvest; there was very little variation in growth rate between seedlings (5). The relative growth rates of different regions of the light-grown leaves increased from the base, reaching a value of 1.0 (i.e., the same rate as that at the tip) 3.0 cm from the base. The growth of etiolated seedlings followed a similar pattern, but was more rapid: tip growth rates rose to 6.2 cm/day, 24 h before harvest, and relative growth rates reached 1.0 by 3.5 cm from the leaf base. From these results, conversion graphs of cell age against distance from the leaf base were computed for 7-day-old light-grown wheat seedlings (Fig. 3) and 5.5-day-old etiolated seedlings. Using these conversion graphs we have been able to compare the plastids in cells of the same age, but in leaves of different ages and grown under different conditions. Etiolated plants gave a curve of the same shape as that for light-grown seedlings, but cells of a given age were farther from the leaf base than in a normally grown seedling.

Plastids of light-grown seedlings synthesize Chl faster as the cell age increases up to 2 days, the rate then remains constant for a further 0.5 days (Fig. 4). This sequence of changes in the ability to synthesize Chl is also seen during the development of wheat etioplasts. The rates of Chl synthesis were obtained by measuring Chl/plastid in leaf sections at different heights from the leaf base of greening etiolated wheat, at regular intervals during 7 h of greening. The maximum rates were plotted against cell age, calculated using the growth rates for dark-grown plants (Fig. 5). There is a marked increase in rate of Chl synthesis per plastid with age during the first days, followed by a declining rate in the oldest plastids. By integration of this curve it was possible to plot for cells of different ages, the Chl/plastid which would be expected if the seedlings could have been grown in light from germination, while still developing their ability to synthesize Chl as they do when grown in darkness (Fig. 5). This is very similar in shape to the curve measured in light-grown seedlings (Fig. 4). Quantitatively, however, there is a large difference between the two systems: the rate of Chl synthesis in etioplasts is about twice that in normally developing chloroplasts of the same age. The conditions of light, humidity and temperature used conventionally, and therefore also in this paper, for growing normal plants, and for greening etiolated material, differ from each other considerably.

Chl a/b Ratios. Marked differences in Chl a/b ratio, measured according to Ogawa and Shibata (13), were found when the two developmental systems were compared. The Chl a/b ratio in light-grown wheat leaves is highest in the youngest cells and falls to a value of 4.48 ± 0.03 by 2.25 days (Fig. 6); this same ratio was also found in more mature tissue. By contrast, plastids from cells of the same age in greening etiolated wheat leaves contain Chl with much higher a/b ratios during the early stages of greening. During the first 2 h of greening, no Chl b could be detected in plastids from any region of the leaf, and only after 8 h did the Chl a/b ratio equal that of light-grown chloroplasts of the same age (Fig. 6). An identical pattern of changes in Chl a/b ratios in both light-grown and etiolated systems was found using Arnon’s method (1) for Chl assay, but the ratios obtained were always lower than the corresponding values determined according to Ogawa and Shibata (13). The Chl a/b ratio measured in mature, light-grown leaf tissue was 3.4 ± 0.1 by Arnon’s method.

Fig. 2. Average contents of Chl/plastid along leaves of 7-day-old light-grown wheat. Points are averages of four separate experiments performed on different batches of seedlings and bars show SE.
wheat seedlings. Features found to be common to both systems, in cells of the same age, must be independent of light. It seems that a plastid has the potential to synthesize Chl at an increasing rate as it becomes older, and that this aspect of its development is independent of light. This potential does, of course, remain latent in the plastids of etiolated leaves until they are illuminated.

Robertson and Laetsch (14) and Mackender (11) reached similar conclusions about the greening of etiolated leaf tissue. Our results extend these observations, and show that the increased greening potential measured by these authors in zones of increasing age in etiolated leaves, is caused largely by an increasing potential rate of Chl synthesis/plastid, and not merely by an increase in the number of plastids/g fresh weight of leaf.

**FIG. 4.** Average contents of Chl/plastid in cells of different ages, in the leaves of 7-day-old light-grown wheat (●–●). These values were derived from those in Figure 2, using the conversion graph in Figure 3. Bars show SE for four experiments. (-----): Rate of Chl formation/plastid at different cell ages, calculated from the slopes of the solid line.

**FIG. 5.** Average maximum rates of Chl formation/plastid in cells of different ages, in leaves of 5.5-day-old greening etiolated wheat, measured over 7 h of greening (●–●). This curve was derived by measuring maximum rates of Chl synthesis/plastid in regions at different distances from leaf base; average age of cells in each region was determined using a conversion graph similar to that in Figure 3, but based on the measured growth rates of etiolated wheat seedlings. (Rates of Chl synthesis/plastid were measured by harvesting sections of leaf at intervals during greening, and plotting the Chl/plastid against time of greening for each region.) Bars show SE for four experiments. Integration of this dashed curve gave the content of Chl/plastid which would have been expected in cells of different ages, if seedlings could have been grown in light from germination, while still developing their ability to synthesize Chl as if grown in darkness (—–).
A feature of the greening of etiolated material which has been well characterized is the rapid decrease in Chl \(a/b\) ratio during illumination, resulting in a ratio characteristic of mature, light-grown tissue after only a few hours light (4, 9). In the present study, every region examined in greening etiolated wheat leaves showed a rapid decrease in Chl \(a/b\) ratio, and, after 8 h greening, each region reached the ratio found in light-grown tissue of the same age.

The elevated Chl \(a/b\) ratios in the youngest cells of light-grown wheat contrast with the results reported for light-grown maize (3), in which the ratio remained constant throughout the leaf, even though the development of photosynthetic activity resembled that of greening etiolated systems. Baker and Hardwick (2) described a model for the development of photosynthetic units in which the Chl \(a/b\) ratio of an individual unit decreased as the unit increased in size. Perhaps this model is applicable to wheat but not to maize.

It is clear that some important plastid properties develop at the same rate in both etioplasts and chloroplasts: the plastids have the potential to synthesize Chl more rapidly as they become older, and the Chl produced has a high Chl \(a/b\) ratio in young plastids, falling to a constant value in cells older than about 2 days. However, 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 this development.

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LITERATURE CITED