Developmental Stages of Cucumber Seedlings

Kinetics of Chlorophyll Accumulation and Other Growth Parameters

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ABSTRACT

The changes in morphology during dark germination and subsequent growth of cucumber (Cucumis sativus) seedlings in the light go through three different phases described as latent, active, and steady-state. This pattern is consistently observed for several related developmental processes. The latent period lasts about 2 days following water imbibition after which the following capabilities appear in concert: (a) root and stem elongation, (b) pigment synthesis including protochlorophyll, chlorophyll, carotenoid, and phytochrome, (c) synthesis of ribulose-1,5-bisphosphate carboxylase/oxygenase, and (d) enhancement of greening by excision. Following the active phase, which lasts for another 2 to 3 days, these processes slow to a steady-state. Inhibition of chlorophyll accumulation by SO₂ was only observed for seedlings in the steady-state phase.

The seed is a potential plant in a dormant state which regains its biological functions after imbibing water, but it is many hours after imbibition before photosynthetic activity can be detected in the germinating seedling. The ontogenesis of the photosynthetic apparatus has been the subject of many investigations, most of them done with dark grown plants which are allowed to green by exposure to continuous white light (24, 25).

It was found that dark grown mustard seedlings do not accumulate Chl at the beginning of germination (11). When older etiolated seedlings were allowed to green, the pattern of Chl accumulation varied with age of seedling in barley (1), cucumber (7, 20), mustard (12), and bean (21). Developing cucumber seedlings from 2 to 7 d after wetting have been exposed to light for greening studies (3, 5-9, 13, 17). The objective of the present study was to study the pattern of age-dependent development of the photosynthetic apparatus in cucumber seedlings, to see how it matches the three phases of structural development (latent, active, and steady-state).

MATERIALS AND METHODS

Plant Material

Cucumber (Cucumis sativus) seeds were donated either by Zeraim Co., Gedera, Israel (var Elem) or by Neuman Seeds Co., El Centro, CA (var Boston Pickles). No difference was observed between the two varieties in the experimental system. The seeds were kept in the dark at room temperature until germination.

Growth in Dark

For experiments with plants older than 3 d, 30 seeds were put on vermiculite in 5 × 5 cm plastic planters and covered with a 1 cm layer of vermiculite. To start germination the planters were soaked with water, put in trays containing water to a depth of around 3 to 4 cm, and placed in a cardboard box wrapped with two layers of black cloth and allowed to germinate in a growth chamber at 26°C. For experiments with plants younger than 3 d, 30 or 60 seeds were put in 10 or 15 cm diameter plastic Petri dishes on three layers of filter paper. The filter paper was wetted until a small quantity (about 2 mL) of water could be drained when the Petri dish was tilted. The Petri dishes were put in boxes and kept in the dark as described above for the older plants. Excised cotyledons were detached and put in Petri dishes in the same way 24 h before exposure to light for greening.

Greening and Light Sources

For greening experiments plants were exposed in the growth chamber to white light under a battery of 12 fluorescent tubes (Sylvania 215V) with an intensity at plant level of 10,000 erg cm⁻² s⁻¹. A safe light was obtained from a similar white fluorescent tube wrapped with sheets of cellophane: three green, two red, and one blue. No conversion of protochlorophyll to Chl was detected in dark grown cotyledons which stayed under the safe light for 20 min.

Pigment Extraction and Determination

Chls a and b, PChl, and carotenoids were extracted with dimethylformamide (DMF) as previously described (15). Samples were done in triplicate, each sample coming from three cotyledon pairs or three cotyledons each from different plants. PChl and Chl in the various plant tissues were determined in the extract using formulae already described (14).

Carotenoid concentrations were determined from absorbance values at the maximum of 480 nm. This is not the highest maximum in the blue for carotenoids, but the absorb-

1 Abbreviations: PChl, protochlorophyll; rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase.
ance peaks of Chl and carotenoids overlap around 450 nm, where the higher maximum of carotenoids is located. The carotenoid level is expressed in absorbance units per gram fresh weight from calculations of the partial absorbance (14) of the maxima at 480 nm, \( A'_{480\text{nm}} \). The determination of \( A'_{480\text{nm}} \) was made using the absorbance values at the two end points of the reference line (510, 469 nm) and at the maximum (480 nm) using the following reference-line equation previously described (14):

\[
A'_{480\text{nm}} = (A_{480\text{nm}} - A_{510\text{nm}}) - (A_{469\text{nm}} - A_{510\text{nm}}) \times (30/41)
\]

If the absorbance is adjusted to zero at 510 nm the equation simplifies to:

\[
A'_{480\text{nm}} = A_{480\text{nm}} - 0.73 \times A_{469\text{nm}}
\]

Relative phytochrome concentrations were determined from the absorbance difference between 806 nm and 726 nm as previously described (18).

**Rubisco Determination**

The amount of rubisco protein present in developing seedlings was measured by a radial immunodiffusion method, employing antisera raised against recrystallized enzyme as previously reported (23). The specific activity was measured as uptake of \(^{14}\)CO\(_2\) from NaH\(^{14}\)CO\(_3\) in the presence of 0.4 mM ribulosebisphosphate (22). Samples were taken daily during 6 d following germination. The samples were taken from six cotyledon pairs that were excised, weighed, and kept in the dark at \(-70^\circ\text{C}\) until further handling.

**Other Methods**

Greening under SO\(_2\) was done in a glass-bell jar. The gas was produced from the reaction of H\(_2\)SO\(_4\) on Na\(_2\)SO\(_3\). The final SO\(_2\) level under the bell jar was 0.025% (v/v).

**RESULTS**

The structural development of cucumber seedlings involves drastic changes starting at the second day of germination (Fig. 1). During the first 2 d there is no significant difference between light and dark grown seedlings, but light plays a
crucial role beginning on the 3rd d in this developmental process. At this time a rapid growth of the root system starts which in darkness ceases at the 4th d. Concurrently, a rapid elongation of the stem begins, which slows down only at the 5th and 6th d and remains stable until around d 10, at which time the stem length is 30 to 35 cm. In the light, root development begins on the 3rd d and continues through d 7. During this period there is less stem elongation as the cotyledons undergo greening and leaf development begins. The rate of Chl accumulation in etiolated seedlings following an initial 4 h exposure to light was measured daily during the first 6 d of dark germination. This period of exposure was chosen to avoid the lag period in the formation which exists for the first 5 d in the dark (24).

Figure 2 shows the total growth and daily growth rate of the stem in the dark. Also shown are Chl accumulation rates observed upon exposure of the cotyledons to light as well as the daily change in the accumulation rate. The processes show generally similar kinetics, which the maximal rate of Chl accumulation occurring a day earlier than the maximal growth rate. Only small changes for both of these parameters were observed during the first 2 d after imbibition of water. Three different phases can be observed for both stem elongation and Chl accumulation: slow (d 1–2), accelerated (d 3–4), and a final decrease to a constant rate (d 5–6). These phases are designated the latent, active, and steady state phases, respectively.

The accumulation rates of nonchlorophyllous pigments, along with phytochrome, were studied for varying periods of dark germination (Fig. 3). The results showed a slow (phytochrome and carotenoids) or zero (PChl) accumulation during the first 2 d, followed by a dramatic increase around 50 h in
the dark following water imbibition. After 75 h the level of phytochrome slowly decreased while that of the two photosynthetic pigments remained fairly constant.

Whereas the data shown in Figure 2 above were obtained for a 4 h illumination period, the effect of the developmental stage of the seedling on Chl accumulation during an initial illumination period was also studied (Fig. 4). As expected, 1 d old seedlings grown in the dark formed no Chl in a subsequent 4 h illumination period. However, 3 d old seedlings were in the active development stage, both for stem elongation and pigment accumulation in the dark, and these responded immediately to the light.

Another indication that all growth parameters are greatest after 3 d of dark germination is the regeneration of the PChl pool after an initial photoconversion of existing PChl to Chl by a flash of light. As shown in Figure 5, the seedlings grown 3 d in the dark gave the most rapid regeneration of PChl. The 6 d seedlings showed the typical lag period. The 1 d seedling produced no PChl.

Another important event in the early development of plants is the formation of the enzyme rubisco. Concentrations of the enzyme protein were determined by an immunological assay and specific activities by an assay for enzyme activity (fixation of $^{14}$CO$_2$). The data show that formation of the enzyme in the cotyledons in the dark as well as development of activity also follow the pattern observed for pigments, with a 2 d delay before accumulation of the enzyme starts. (Fig. 6). For rubisco formation, however, the protein level and enzyme activity seems to level off somewhat later.

Two treatments which are known to affect greening were studied in the different phases of seedling germination. It is known that pigment accumulation in cotyledons detached with their hooks is slower than that observed for cotyledons detached without hooks (16). The effects on Chl formation of excision of the roots of developing seedlings, with and without the hook remaining, are shown in Figure 7. For this experiment seedlings at 3 and 6 d of development were used, since both are able to form Chl upon illumination. A higher rate of Chl formation was observed for the 6 d plants without the hook. Excision of the hook had no effect on the 3 d plant.

The gas SO$_2$ is a common air pollutant and has also been
shown to affect greening (2). Its effect on Chl accumulation was observed with 3 and 6 d old plants, which are competent for greening. Only the 6 d plants showed an inhibition of Chl accumulation due to SO$_2$ (Fig. 8).

**DISCUSSION**

Three distinct periods can be defined in the structural development of cucumber seedlings (Fig. 1). This triphasic pattern appears to be significant since it reflects a similar pattern of development in the physiology of germination, including pigment changes and rubisco formation (which leveled off somewhat later) during ontogenesis of the photosynthetic apparatus. These phases are not a function of illumination, since they are observed during germination in the dark for all the parameters we have studied, including the formation of pigments upon exposure to light.

The latent phase lasts for 2 d after water imbibition. During this period the radicle and the hypocotyl hook are forming. Even under light there is little or no accumulation of pigments, including PChl, or rubisco.

The second period is the active phase, which involves rapid stem elongation at a rate reaching 2 mm/h. During this period the root system is also significantly developed. The second period also involves pigment synthesis and rubisco formation. There is immediate Chl and PChl accumulation following exposure to light and the rate of accumulation increases during this phase. This period lasts about 2 d, through the 3rd and 4th d after imbibition.

The steady state phase begins around the end of the 4th d. In this phase all processes slow down and enter into a steady state. The PChl and carotenoids are maintained at a low level. There is a slow-down and then a stop of hypocotyl elongation. No other structural changes occur except the 'straightening' of the hypocotyl-hook.

Our findings on the accumulation of PChl and carotenoids during germination of cucumber seedlings in the dark, determined with several hour intervals, are in agreement with results obtained by others with 1 d intervals for PChl (19) and carotenoids (20). Similar results were obtained for PChl in bean (4). It is now clear that the kinetics of greening are different in the three phases: zero Chl accumulation at the first phase, immediate accumulation at the second phase, and the common, 'lag before greening' pattern through the third phase (24). Treatments which enhance greening, such as excision (16), or treatments which inhibit, such as SO$_2$ (2, 10), confirm the difference in the physiology of the different phases. It is obvious that consideration of the developmental phase of the seedling (latent, active, or steady-state) is very important for any study of the ontogenesis of the photosynthetic system.

During the latent phase no greening takes place since the plant has no capacity for it. During the active phase the plant has a high metabolic rate and a high capacity for greening, which means that ontogenesis of the photosynthetic apparatus may be studied during this phase. However, at this phase other developmental processes are also changing at high rates regardless of light conditions. Results obtained with probes intended to study greening are liable to be obscured or modified either directly by the interaction of other processes with the greening process or indirectly by the effect of the probes on the other processes or by both. This could complicate the analysis of the results and should be seriously considered when developing the experimental protocol.

During the steady state phase (around 6 d in cucumber) developmental processes slow down to a relatively stable state, minimizing the effects of other processes which occur during the active phase. This makes the steady state phase the best for experiments intended to study the mechanism of greening in etiolated tissues from dark grown seedling.
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LITERATURE CITED