THE INFLUENCE OF LIGHT OF DIFFERENT SPECTRAL REGIONS ON THE SYNTHESIS OF PHENOLIC COMPOUNDS IN GHERKIN SEEDLINGS, IN RELATION TO PHOTOMORPHOGENESIS

VI. PHENOL SYNTHESIS AND PHOTOPERIODISM

G. ENGELSMA

Philips Research Laboratories, N.V. Philips' Gloeilampenfabrieken, Eindhoven-Netherlands

SUMMARY

In the hypocotyl of dark-grown gherkin seedlings a series of maxima in the level of phenylalanine deaminase (PADAse), a key enzyme in phenol synthesis, can be induced by an alternating light-dark treatment. Each PADAse peak corresponds to a temporary increase in the rate of accumulation of hydroxycinnamic acids. The height of a particular enzyme peak is a function of the length of the inducing light period and of the light sensitivity of the plant at the time of irradiation. The light sensitivity is inversely related to the amount of hydroxycinnamic acids that have previously accumulated in consequence of preceding irradiation. Possible implications of these findings on the problem of biological time measurement are discussed.

1. INTRODUCTION

In the leaves of *Kalanchoe blossfeldiana* the biosynthesis of anthocyanin occurs only under conditions which also lead to flowering (NEYLAND *c.s.* 1963). This has raised the question whether initiation of flowering would be causally related to the induction of phenol synthesis. In more recent investigations by TAYLOR (1965) and ZUCKER *c.s.* (1965), who studied the effect of photoperiod on the biosynthesis of chlorogenic acids in respectively *Xanthium* and *Nicotiana*, such a relationship could not be established. Therefore it may well be that the two photoresponses are realized via entirely different chains of events. On the other hand, as will be seen in the present paper, there are certain points of correspondence between the way in which the system controlling phenol synthesis and that responsible for flower initiation respond to alternating light and dark periods. This may indicate that the same regulatory principles are involved. The relative temperature independence which is achieved with the mechanism that controls phenol synthesis in gherkin seedlings has already been discussed (ENGELSMA 1968b). In the present paper it is pointed out how, on the basis of the principles of this mechanism, different aspects of biological time measurement, as observed in photoperiodic phenomena, might be interpreted.

2. MATERIAL AND METHODS

All experiments were carried out with gherkin seedlings (*Cucumus sativus* L., "Venlose niet plekkers", strain Tercken VI) grown in darkness at 25°C. Three

days after sowing, when the seedlings had reached a length of 3 cm, they were placed in cabinets maintained at 18 °C in which they were subjected to different irradiation programs. Blue light of 150 $\mu W/cm^1$ obtained by the light and filter combination as described by MEYER (1957) was used. The quantitative determination of hydroxycinnamic acids was performed as described by ENGELSMA & MEIJER (1965) from duplicate samples of 20 hypocotyls each. Phenylalanine deaminase (PADAse) was extracted and assayed as described by ENGELSMA (1967a) from samples of 100 hypocotyls each.

3. RESULTS AND DISCUSSION

3.1. The influence of alternating light and dark periods on the development of PADAse and the accumulation of phenols

A series of maxima in the level of PADAse, a key enzyme in phenol synthesis, can be produced in gherkin hypocotyls by an alternating light-dark treatment of the seedlings (*fig. 1*). In order to obtain enzyme peaks of equal heights each successive light period has to be longer than the preceding one (*fig. 1D*). *Fig. 1C* shows that if the daily light period is of a constant length the second maximum is much lower than the first one and that no third maximum is obtained. These findings demonstrate a gradual loss of light sensitivity, a phenomenon first observed when the seedlings were subjected to irradiation programs with different light intensities (ENGELSMA 1967a). This decrease in light sensitivity is not a matter of the seedlings becoming older: five-day-old dark-grown seedlings give about the same response as three-day-old dark-grown seedlings (*fig. 1B*). It must therefore be due to processes induced by the preceding irradiation, and a mechanism suggested previously (ENGELSMA 1967a) is that the end products (hydroxycinnamic acids), which accumulate in the cells where they are produced, cause repression of PADAse synthesis. The finding that p-coumaric acid inhibits the development of PADAse in hypocotyl segments supports this supposition (ENGELSMA 1968a).

Fig. 2 shows that each peak in the PADAse level corresponds to a temporary increase in the rate of accumulation of hydroxycinnamic acids, and from a comparison with *fig. 1* it appears that, in agreement with the above hypothesis, there is an inverse correlation between the level of hydroxycinnamic acids already present and the light sensitivity, as far as induction of PADAse is concerned.

3.2. Aspects of time measurement

In *fig. 1* it can be seen that irrespective of the duration of irradiation the time between the beginning of a light period and the moment when the PADAse level reaches a maximum is always about the same. A comparison of curves A, C_1 and D_1, and of curves D_2 and D_2', shows that the peak height is a function of the length of the inducing light period and of the light sensitivity of the plant at the moment of irradiation. The same applies to the induced increases in the level of hydroxycinnamic acids.

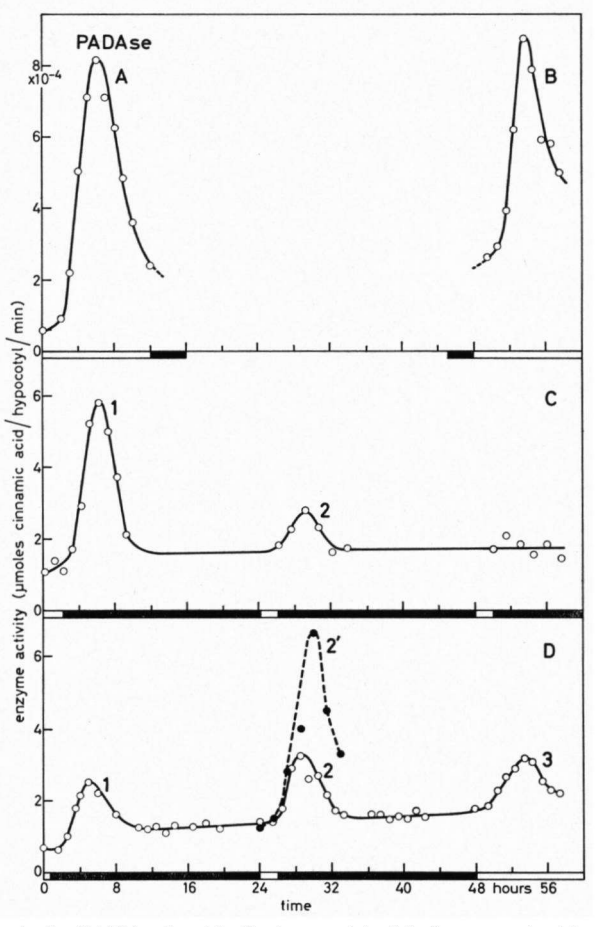

Fig. 1. Changes in the PADAse level in the hypocotyl of dark-grown gherkin seedlings which 72 hours after sowing were treated as follows: A: 12 hours light, followed by darkness; B: 48 hours darkness, 12 hours light; C: 2 hours light, 22 hours darkness, 2 hours light, 22 hours darkness, 2 hours light, 10 hours darkness; D: 50 min light, 23 hours darkness, 2 hours light, 22 hours darkness, 12 hours light.
Curve D_2' refers to seedlings which after a pretreatment of 50 min light and 23 hours darkness were continuously irradiated.
Light: blue, 150 μW/cm². Temperature: 18 °C.

Let us now discuss whether it is possible to correlate these findings with photoperiodic induction. The question can be asked how a regulatory mechanism, as advanced for photoinduced phenol synthesis, would function if the end product, instead of being stored in the cell where it is produced, were transported to another part of the plant, as is apparently the case with the flower hormone. In the first place this modification opens the possibility that, as far as end-product repression is concerned, the enzyme synthesizing system is in the

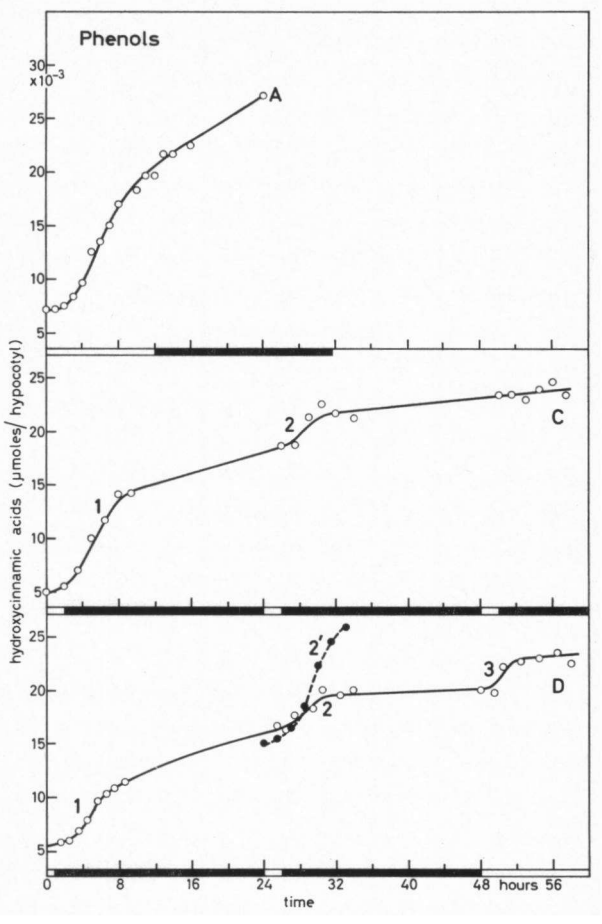

Fig. 2. Accumulation of hydroxycinnamic acids in the hypocotyl of dark-grown gherkin seedlings which 72 hours after sowing received respectively the treatments A, C, and D mentioned in *fig. 1.*

same state at the beginning of each successive light period. The relation between the lengths of the light periods and the heights of the respective enzyme maxima would then remain the same during the whole irradiation program and each light period would find its length reflected in the amount of end product whose synthesis it had induced. In the second place, the modification of our system implies that the initial increase in end-product repression is followed by a decline and this might give rise to oscillations in the enzyme level, as have, for instance, been observed with certain hormone-induced enzymes in animal systems (WURTMAN & AXELROD 1967; TSCHUDY *c.s.* 1967). A single light period could thus induce more than one enzyme maximum. To these changes in enzyme level would correspond changes in the end-product concentration and the resulting oscillations in the repression of enzyme synthesis would appear as

alternating light-sensitive and light-insensitive periods. The response will now depend on whether the light-dark pattern matches these periods. Thus two important similarities to photoperiodic phenomena arise. These are: (1) the relation between the response and the length of the light period, and (2) the phenomenon that a response follows only those irradiations that occur at certain time intervals (see SALISBURY 1963).

Another corresponding feature between photoinduced phenol synthesis and a photoperiodic phenomenon like flower initiation is the relative independence of light intensity and temperature. Both in the blue and far-red regions light intensities of about 100μW/cm^2 have been found to be saturating with respect to the induction of PADAse in gherkin seedlings (ENGELSMA 1967a, b). For light intensities beyond this level the response depends solely on the duration of irradiation. The relative temperature independence of photoinduced phenol synthesis has been pointed out in the preceding paper (ENGELSMA 1968b). The most interesting aspect, as far as correlation with photoperiodic phenomena is concerned, is revealed by *fig. 3* of that paper, where it is shown that in the case of light-dark treatments there is a particular "critical" light period characterized by the fact that, over a fairly wide temperature range, shorter light periods always cause the production of a smaller amount of phenols, whereas longer light periods always induce the synthesis of a greater amount.

Finally it should be noted that, as far as light requirement is concerned, the analogy of photoperiodic induction of flowering with photoinduced phenol synthesis is restricted to long day plants. Short-day behaviour might be explained, however, on the basis of the same regulatory principles if the assumption were made that in that case the synthesis of a key enzyme is repressed by irradiation. Such enzymes may well exist (WOLF 1968). The way in which light and temperature affect their development is a subject meriting further investigation.

ACKNOWLEDGEMENT

The author wishes to express his thanks to Miss M. C. Braun, Miss E. M. Linckens, and Mr. J. M. H. van Bruggen for technical assistance.

REFERENCES

ENGELSMA, G. (1967a): Photoinduction of phenylalanine deaminase in gherkin seedlings. I. Effect of blue light. *Planta (Berl.)* **75**: 207–219.

– (1967b): Photoinduction of phenylalanine deaminase in gherkin seedlings. II. Effect of red and far-red light. *Planta (Berl.)* **77**: 49–57.

— (1968a): Photoinduction of phenylalanine deaminase in gherkin seedlings. III. Effects of excision and irradiation on enzyme development in hypocotyl segments. *Planta (Berl.)* **82**: 355–368.

— (1968b): The influence of light of different spectral regions on the synthesis of phenolic compounds in gherkin seedlings, in relation to photomorphogenesis. V. The temperature dependence. *Acta Bot. Neerl.* **17**: 499–505.

— & G. MEIJER (1965): The influence of light of different spectral regions on the synthesis of

phenolic compounds in gherkin seedlings, in relation to photomorphogenesis. I. Biosynthesis of phenolic compounds. *Acta Bot. Neerl.* **14**: 54–72.

MEIJER, G. (1957): The influence of light quality on the flowering response of Salvia occidentalis. *Acta Bot. Neerl.* **6**: 395–406.

NEYLAND, M., YUK LIN NG & K. V. THIMANN (1963): Formation of anthocyanins in leaves of Kalanchoe blossfeldiana: a photoperiodic response. *Plant Physiol.* **38**: 447–451.

SALISBURY, F. B. (1963): *The flowering process.* Pergamon Press. Oxford, London, New York, Paris, 234p.

TAYLOR, A. O. (1965): Some effects of photoperiod on the biosynthesis of phenylpropane derivatives in Xanthium. *Plant Physiol.* **40**: 273–280.

TSCHUDY, D. P., A. WAXMAN & A. COLLINS (1967): Oscillations of hepatic δ-aminolevulinic acid synthetase produced by estrogen: a possible role of "rebound induction" in biological clock mechanisms. *Proc. Nat. Acad. Sci.* **58**: 1944–1948.

WOLF, F. T. (1968): Enzymatic activities in the leaves of light-grown and dark-grown wheat seedlings. *Z. Pflanzenphysiol.* **59**: 39–44.

WURTMAN, R. J. & J. AXELROD (1967): Daily rhythmic changes in tyrosine transaminase activity of rat liver. *Proc. Nat. Acad. Sci.* **57**: 1594–1598.

ZUCKER, M., C. NITSCH & J. P. NITSCH (1965): The induction of flowering in Nicotiana. II. Photoperiodic alteration of the chlorogenic acid concentration. *Am. J. Bot.* **52**: 271–277.