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

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SUMMARY

The increase in the rate of synthesis of hydroxycinnamic acids in response to irradiation is greater at higher temperatures, but the length of the period of enhanced accumulation is shorter. This can be explained by the influence of the temperature on the photoinduced changes in the level of the enzyme phenylalanine deaminase (PADAse): at higher temperatures the enzyme increases more rapidly but the subsequent decline of its level begins sooner. In the range of 12.5 to 32 °C the lower temperatures are optimal for phenol synthesis if the seedlings are continuously irradiated. In the case of irradiation programs of a light period followed by darkness a maximum amount of phenols is synthesized at higher temperatures: the shorter the inducing light period, the higher is the optimal temperature. It is shown that there is one particular combination – 2.5 hours of light followed by 21.5 hours of darkness – with which the apparent Q_{10} for phenol production is close to unity in the whole temperature range of 18 to 32 °C.

1. INTRODUCTION

Temperature has an accelerating effect on chemical reactions, whether catalysed or uncatalysed. The value of the temperature coefficient (Q_{10}) is usually at least 2. Certain phenomena in plants may, however, be less temperature sensitive and sometimes show Q_{10}'s equal, or close, to unity. Photoperiodic induction of flowering is an example of this (see LANG 1965). The explanation given for the apparent low Q_{10} is that the response would be determined by an interaction of promotive and inhibitory processes in which temperature-caused differences would tend to cancel each other out. In this respect a certain analogy may exist with the photoinduction of phenol synthesis in gherkin seedlings, a phenomenon that is determined by the enzyme phenylalanine deaminase (PADAse) whose level is regulated by processes that antagonize each other, namely induction of PADAse synthesis on the one hand, and inactivation of this enzyme and repression of its synthesis on the other (ENGELSMA 1967a, b; 1968a). The influence of the temperature on these processes can be determined, and therefore this system seems to be well suited for an analysis of the temperature dependence of a particular photomorphogenetic response.

2. MATERIAL AND METHODS

For all experiments three-day-old gherkin seedlings (*Cucumus sativus* L., "Ven-

lose niet plekkers", strain Tercken VI) were used, raised at 25 °C in the dark (ENGELSMA & MEYER 1965). The irradiations were carried out, at the temperatures indicated, in cabinets with blue light of 150 $\mu W/cm^2$ obtained with the light and filter combination as described by MEYER (1957). In order to allow the seedlings to adapt themselves to the different temperatures they were placed in the respective cabinets at least 30 min prior to the onset of the irradiation. The quantitative determination of hydroxycinnamic acids was performed as described by ENGELSMA & MEYER (1965) from duplicate samples of 20 hypocotyls each. PADAse was extracted and assayed as described by ENGELSMA (1967a) from samples of 100 hypocotyls each.

3. RESULTS AND DISCUSSION

3.1. The dependence on temperature of the changes in the PADAse level and in the phenol synthesis in dark-grown seedlings that are continuously irradiated

Exposure of dark-grown gherkin seedlings to light causes a temporary rise in the rate of accumulation of hydroxycinnamic acids in the hypocotyl (ENGELSMA & MEYER 1965). The enhanced accumulation is preceeded by a time lag, continues for a certain length of time, and is then followed by a phase during which the rate declines to more or less the same value as before the irradiation. For seedlings which from a certain moment are continuously irradiated, *fig. 1* shows that at lower temperatures both the lag phase and the period of enhanced phenol synthesis are longer and that the rate of this synthesis is smaller. This influence of the temperature on the time course of phenol synthesis can be directly explained from the curves that for different temperatures show the changes induced by light in the PADAse level in gherkin hypocotyls *(fig. 2)*. For these changes, too, the rule holds that at lower temperatures the time lag is longer and that the rate of the subsequent increase in the enzyme level is smaller. This means that at lower temperatures the rate of the induced phenol synthesis will be smaller, the more so as it may be expected that at lower temperatures the rate of the reaction catalysed by PADAse will be lower. On the other hand, the lower the temperature, the longer the time becomes which is needed to reach the moment at which the enzyme level begins to decline. This leads to a higher PADAse level which is maintained for a longer period and explains why at lower temperatures the enhanced phenol synthesis continues for a greater length of time, resulting in an overcompensation for the decline in the rate of accumulation. It thus appears that for the range from 12 to 32 °C the lower temperatures are optimal for phenol synthesis in seedlings that are continuously irradiated.

3.2. The effect of temperature on phenol synthesis as a function of the length of the inducing light period

In experiments performed at 25 °C it has been shown previously (ENGELSMA & MEYER 1965; ENGELSMA 1967a) that if gherkin seedlings are irradiated for a

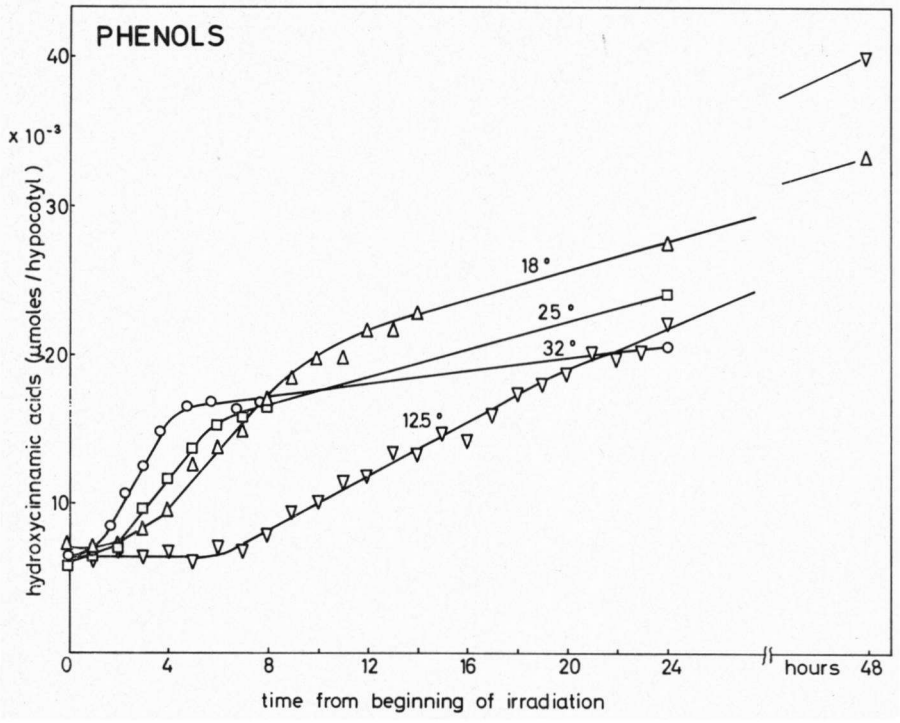

Fig. 1. The influence of the temperature on the accumulation of hydroxycinnamic acids in the hypocotyl of three-day-old, dark-grown gherkin seedlings that are continuously irradiated with blue light of 150 $\mu W/cm^2$.

certain time and subsequently kept in darkness, the total amount of hydroxycinnamic acids synthesized in the course of 24 hours increases with the irradiation time up to a certain point. This limiting value is of the same order as the time needed for PADAse to reach its maximum. Apparently irradiation that continues beyond the attainment of the enzyme maximum and thus falls into the period in which inactivation of PADAse predominates over its synthesis, progressively becomes less effective in inducing PADAse synthesis and therefore contributes increasingly less to phenol synthesis. This has been the main argument for the supposition that concomitant with the inactivation of PADAse its synthesis becomes repressed.

Comparison of *figs. 2* and *3* confirms that over the whole temperature range from 12 to 32 °C such a correlation exists between the time needed for PADAse to reach its maximum and the length of the light period in which the phenol synthesis reaches saturation. The lower the temperature, the longer these periods become. *Fig. 3* shows that a short period of irradiation is more effective if given at a higher temperature. On the other hand, as we have already seen in the preceding section, lower temperatures are optimal if the seedlings are continuously irradiated. The curves which for different temperatures show the

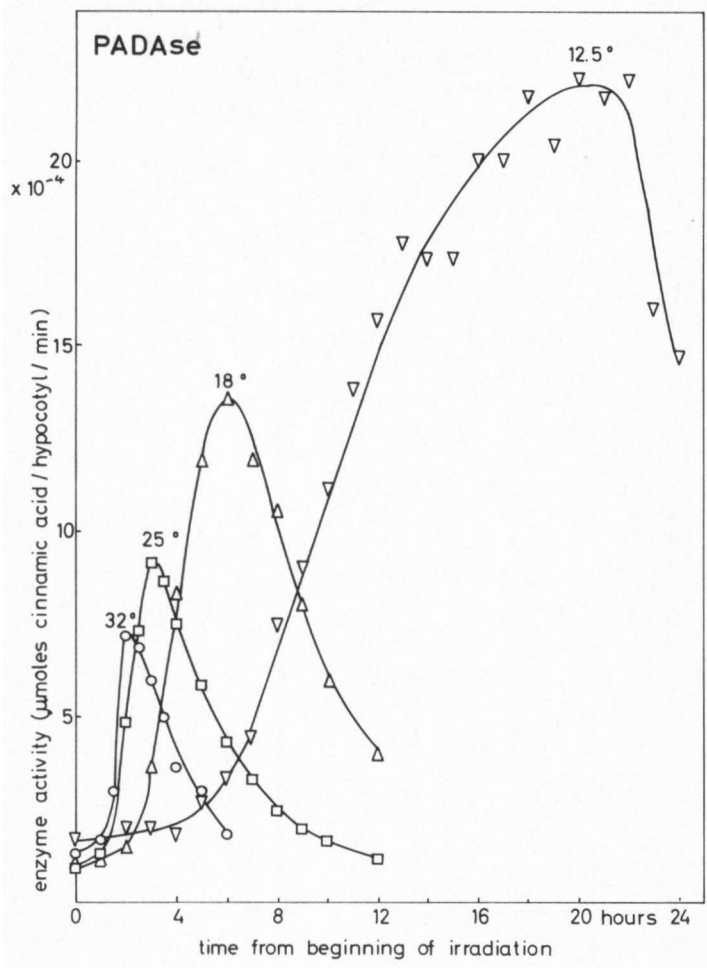

Fig. 2. The influence of the temperature on the changes in the level of PADAse in the hypocotyl of three-day-old, dark-grown gherkin seedlings that are continuously irradiated with blue light of 150 μW/cm².

amount of hydroxycinnamic acids synthesized in the course of 24 hours as a function of the duration of irradiation, will thus intersect. The most notable aspect revealed by *fig. 3* is that the points of intersection of the curves for 18, 25, and 32 °C nearly coincide, which means that under a certain condition – 2.5 hours of light followed by 21.5 hours of darkness – the apparent Q_{10} for phenol production is close to unity over a temperature range of at least 14 °C. Irrespective of the temperature within the above range we find that light periods shorter than 2.5 hours will always cause the production of a smaller amount of

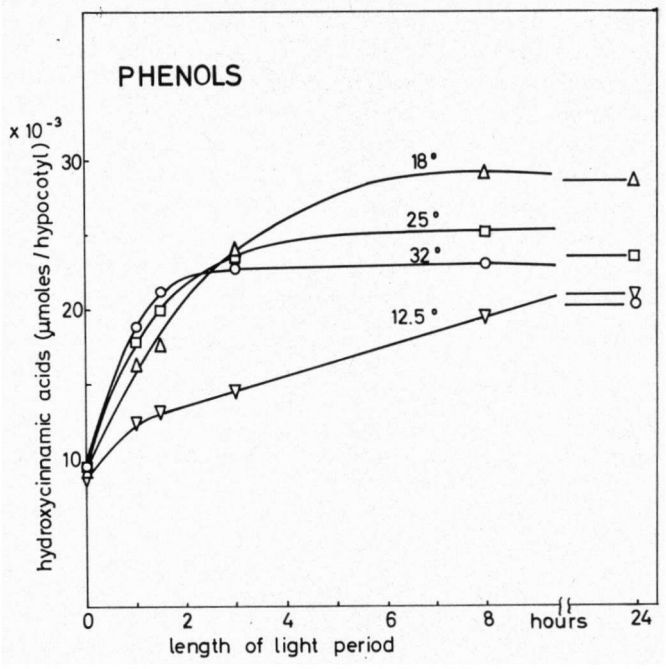

Fig. 3. Accumulation of hydroxycinnamic acids in the gherkin hypocotyl during 24 hours following the beginning of irradiation as a function of the duration of irradiation and of the temperature. Light: blue, 150 µW/cm².

phenols than induced by a 2.5–hour light period, whereas longer light periods will always induce the synthesis of a greater amount.

3.3. The influence of temperature on anthocyanin synthesis

As far as we know, the influence of temperature on phenol synthesis has hitherto been studied only in connection with anthocyanin synthesis. Since anthocyanin formation depends on cinnamic acid synthesis, PADAse could have a regulatory function in this process, too. From the curves of the time dependence of the photoinduced changes in PADAse as presented by DURST & MOHR (1966a, b) and SCHERF & ZENK (1967a, b) for respectively mustard and buckwheat seedlings, it may be concluded that in other plants the PADAse level is regulated in a similar manner as in gherkin seedlings. Therefore the temperature might be expected to influence anthocyanin synthesis in a similar way as it affects the synthesis of hydroxycinnamic acids. The results of most of the earlier workers, who found that low temperatures have a favourable influence on anthocyanin formation, appear to confirm this expectation (see BLANK 1958). It is also consistent with results of TROYER (1964) who reported that in buckwheat seedlings the peak amount of pigment at 10° was greater than at 25 °C. Two exceptions have appeared in the literature. FREY-WYSSLING & BLANK (1945) found that in seedlings of red cabbage the anthocyanin content was

much higher at 20° and 30 °C than at 10 °C. It should be noted, however, that these authors studied the development of the pigment in darkness and it may well be that in that case the optimal temperature for anthocyanin formation coincided with that for the metabolism in general. SIEGELMAN & HENDRICKS (1958) reported that in isolated slices of apple skin a greater amount of anthocyanin was formed at higher temperatures. The explanation for this difference in temperature dependence may lie in the fact that the photosynthetic system plays a role in anthocyanin synthesis in apple skin (DOWNS *c.s.* 1965) whereas in seedlings it does not (BERTSCH & MOHR 1965). Moreover, slicing may seriously disturb the regulatory system that controls the PADAse level, as has been shown in gherkin seedlings (ENGELSMA 1968b).

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