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Short Communication

Phytochrome-mediated Flavone Glycoside Synthesis in Cell Suspension Cultures of *Petroselinum hortense* after Preirradiation with Ultraviolet Light

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Summary. Ultraviolet light was demonstrated to stimulate flavone glycoside synthesis in *Petroselinum* cell suspension cultures. The data presented suggest the involvement of phytochrome in this response: Flavone glycoside formation resulting from 1 h of ultraviolet irradiation was increased by subsequent continuous far-red light irradiation. However, the ultraviolet effect was reduced by a sub-sequent irradiation with 10 min of far-red. This far-red effect was fully reversed by a sub-sequent irradiation with 10 min of red. Red and far-red irradiations were ineffective without ultraviolet preirradiation. It is concluded that in this system ultraviolet irradiation is required in order to change the cells in such a way as to allow a physiological effectiveness of the phytochrome system.

Most enzymes involved in the pathway of flavone glycoside synthesis in cell suspension cultures from parsley (*Petroselinum hortense*) have been identified (Hahlbrock *et al.*). The activity of each of these enzymes, as well as flavone glycoside accumulation, increases drastically upon illumination with white light (Hahlbrock *et al.*).

Since there is little information about photoresponses in cell cultures, except those regarding photosynthesis or growth, an analysis of this light effect was undertaken. One phytochrome effect demonstrated in this context was a slight change in growth rate of callus tissue (Kasperbauer and Reinert). Another well-established light response in cell or tissue cultures, the accumulation of anthocyanin in *Haplopappus*, seems not to be mediated by phytochrome (Lackmann). The finding of a phyto-chrome response involving a well-defined step of differentiation in a cell culture would provide an appropriate model system for investigating the molecular mechanism of cell differentiation.

Cell suspensions from parsley were obtained and grown as described previously (Hahlbrock *et al.*), subculturing every 7 days. For experiments constant amounts of cells from 7 days' old cultures were collected by means of small fritted glass filters and transferred to 5×5 cm glass petri dishes containing constant volumes of medium. The tubes were covered with plastic lids which had good transmission in the near ultraviolet (Falcon Plastics, Los Angeles). Growth of the cultures

in the dark and irradiation experiments were done under sterile conditions with continuous shaking at 25° C.

For irradiation standard red (67.5 $\mu W \cdot cm^{-2}$) (Mohr *et al.*, 1964) and standard far-red (350 $\mu W \cdot cm^{-2}$) (Mohr, 1966) sources were used. White light (ca. 5000 $\mu W \cdot cm^{-2}$) was obtained from a high pressure xenon arc and ultraviolet (75 $\mu W \cdot cm^{-2}$) from ultraviolet lamps (Osram L40W/73, λ_{max} 350 nm).

Extracts were prepared by homogenizing 1 g of cells (fresh weight) in 5 ml 0.1 M borate buffer, pH 8.8, in a Sorvall Omni-Mixer. (Borate buffer was used because phenylalanine ammonia-lyase was measured in the same extracts.) In the 35000 g (15 min) supernatant, the flavone glycoside content was determined by measuring the absorbance at 380 nm, protein by a standard biuret method (Layne) and the activity of glucose-6-phosphate dehydrogenase after Kuby and Noltmann.

As a system of reference the fresh weight was used, since it was not influenced by the irradiation. Proportionality between extract volume and enzyme activity and reaction linearity for 10 min were always observed. Values in Tables 2 and 3 are means of 8-12 parallel and independent experiments. The standard error is always less than 5%.

The spectral range active in inducing flavone glycoside accumulation was estimated by means of a series of glass filters with a sharp transmission cut-off at the shorter wavelengths (Schott, Mainz). For this purpose the incubation vessels were wrapped in aluminum foil so that light could only reach the cells through a window in the lid covered with the filter. The data of Table 1 indicate that maximum quantum efficiency was below 320 nm if one takes into account that the spectral emission of the xenon lamp decreases rapidly in ultraviolet range. Preliminary action spectra using a Bausch and Lomb monochromator are consistent with a maximum quantum efficiency below 300 nm. The visible range of the spectrum, as well as long time irradiations with high intensities of red or far-red light, proved to be without detectable effect (Table 3).

During the time of treatment fresh weight increased to the same amount in either dark-treated or irradiated cultures. Also no differences

Table 1. *Estimation of the spectral range active in flavone glycoside accumulation by means of ultraviolet absorbing filters. The samples were irradiated with white light for 2 h and flavone glycosides measured after a further 20 h of darkness*

Filter used λ_H[a]	Flavone glycosides A_{380}[b]
435	0.13
385	0.19
345	0.31
320	0.73
280	0.90
without	0.92
dark control	0.12

[a] Wavelength at which transmittance of the filter is 50%.
[b] Standard extract = 1 g fresh weight/5 ml buffer.

Table 2. *Test for light-dependent changes in fresh weight, protein content and extractable activity of glucose-6-phosphate dehydrogenase*

Program of irradiation	Fresh weight (g/sample)	Protein (mg/ml extract)	Glucose-6-phosphate dehydrogenase (ΔA/min)
60 min U.V. + 10 min red + 15 h dark	1.41	0.85	0.119
60 min U.V. + 10 min far-red + 15 h dark	1.39	0.88	0.128
60 min U.V. + 15 h far-red	1.42	0.86	0.123
16 h dark	1.37	0.86	0.125

Table 3. *Flavone glycoside accumulation mediated by short-time irradiation with red and far-red light and with continuous far-red light*

Program	Flavone glycosides[a] (A_{280})
Preirradiation with 60 min ultraviolet followed by:	
15 h dark	0.36
15 h far-red	0.41
10 min red + 15 h dark	0.355
10 min red + 10 min far-red + 15 h dark	0.255
10 min far-red + 15 h dark	0.26
10 min far-red + 10 min red + 15 h dark	0.35
Without ultraviolet preirradiation:	
16 h red	0.12
16 h far-red	0.125
10 min red + 16 h dark	0.12
10 min far-red + 16 h dark	0.125
16 h dark	0.125
Initial value before irradiation	0.12

[a] Standard extract = 1 g fresh weight/5 ml buffer.

in protein content due to the irradiation could be detected. The activity of glucose-6-phosphate dehydrogenase remained unchanged. There was no indication of any damage to the cells due to the standard ultraviolet irradiation used. A linear increase in flavone glycoside accumulation was measured with respect to the time of ultraviolet irradiation up to 5 h (measured after 20 h of darkness).

Of special interest is the finding of a second photoresponse detectable only after preirradiation with ultraviolet light. The data of Table 3 indicate the involvement of phytochrome in this response. The stimulation of flavone glycoside accumulation observed after 60 min standard ultraviolet irradiation was reduced by about 40% by a subsequent pulse

of 10 min of far-red light. This reduction of the ultraviolet effect was nullified by a subsequent red irradiation. Red and far-red light given without ultraviolet pretreatment had no effect. The fact that red light given after ultraviolet showed no additional stimulation of flavone glycoside accumulation can be explained by the formation of a large percentage of P_{fr} by the ultraviolet preirradiation.

Another indication for the involvement of phytochrome is the result that continuous far-red light increased flavone glycoside accumulation to values higher than those obtained after ultraviolet followed by darkness (Table 3). It must be emphasized that there was no effect of continuous red or far-red light upon flavone glycoside formation without a preceding irradiation with ultraviolet light.

Though we are unable as yet to distinguish clearly between the two effects of ultraviolet and red or far-red light, the data thus far presented suggest a model system for the study of a double action mechanism in differentiation: ultraviolet light exerts some specific action which changes the cells in such a way that phytochrome becomes able to exert its regulatory influence on the differentiation process (Mohr and Sitte).

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