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The Effects of Daily Exposure to UV-A Light on Phenylalanine Ammonia-Lyase Activity During the Growth of Barley Plants

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The activity of phenylalanine ammonia-lyase (PAL) during the life cycle of barley plants (*Hordeum distichon* L.) exposed to UV-A radiation (355 nm) during 15, 30 and 60 min day^{-1} was studied. In comparison with the control plants, a stimulatory effect on PAL activity was observed. This effect was directly related to the exposure time to UV-A radiation. It was also noted that the amount of protein extracted, decreased significantly with age increase in all treatments. The studied enzyme showed its highest activity during early stages of growth. A sharp and progressive decline in PAL activity was observed in older plants. This decrease was more evident during the development of the ear.

Key words: *Hordeum distichon*, Barley, PAL activity, UV-A radiation.

The enzyme phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) catalyzes the conversion of L-phenylalanine to trans-cinnamic acid, an important precursor of soluble plant phenolic compounds in higher plants. In many plant tissues PAL activity is influenced by various agents, both physical and chemical (7), among which light is one of the most interesting. Since ZUCHER's report (34) on the induction of phenylalanine ammonia-lyase by light in potato tuber tissue, the photoinduction of this enzyme has been studied in a great number of other plants (5, 7, 36). Light produces an increased PAL activity, as reflected by an increase of cinnamate-derived compounds. As it is currently known, taking into account all the experimental data, obtained from different plants, there is no single mechanism of PAL regulation (14, 17). This enzyme responds to white, blue, ultraviolet, red and far-red light. Ultraviolet radiation has been known for a long time

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to produce an increase in PAL and other enzymes involved in phenolic biosynthesis in plants (29). This stimulation of PAL activity is apparently associated with an increase in resistance rather than with UV injury. However, most investigations have focused on the effects produced by UV-B light and less attention has comparatively been paid on the UV-A radiation. The purpose of this paper is to study UV-A radiation effects in PAL activity during the life cycle of barley plants (*Hordeum distichon* L.).

Material and Methods

Culture and irradiation conditions. — The experiment was carried out during the life cycle of barley plants (*Hordeum distichon* L.) during the growing season for this species (April-July). The plants were grown in a greenhouse, at 22° C and 66 % relative humidity. Four sets made up of three pots (30 × 30 × 20 cm) were used. For the experiment, three of them were exposed daily to UV radiation: 15 (treatment T_1), 30 (treatment T_2) and 60 (treatment T_3) minutes each, in addition to an untreated control set. Supplemental UV-A irradiance (8.8 W m^{-2}) was provided by light fixtures each containing five ultraviolet lamps (Sylvania F20T12, 320-400 nm, λ_{max} 355 nm) as described previously (4). Fixtures were placed 40 cm above the plants. This distance was kept constant during the plant growth. The irradiation treatment started the day after sowing. All plant material was harvested every 7 days, following the irradiation treatment and prior to daily watering at the same time of day (10:00 a.m.) to ensure comparable conditions. At the end of the experiment, 17 samples of each of the following sets were obtained: control and treatments T_1 and T_2, and only 14 samples of treatment T_3. Table I shows the time of irradiation, in minutes, and the age of plants, in days.

Phenylalanine ammonia-lyase extraction and assay. — The plant material (aerial part) was homogenized in borate buffer, (0.1 M, pH 8.8), in a Sorval Omni-Mixer (2 min, top speed). In order to avoid phenolic material that could interfere the spectrophotometric assay, the grinding medium included 2-mercaptoethanol (5 mM) and insoluble PVP (10 %, w/v). The extract was collected after centrifugation at 29,200 × *g* for 30 min in a Beckman J-21 centrifuge at 4° C. The supernatant was filtrated through Sephadex G-25 columns in order to avoid possible errors in quantitative determination (10). The resulting extract was used for the enzyme preparation. All the above operations were carried out at 4° C.

PAL activity was assayed by the spectrophotometric method described by ZUCKER (34). The incubation mixture contained 1 ml of borate buffer (0.1 M, at pH 8.8), 1 ml of L-phenylalanine (60 mM) dissolved in buffer and 1 ml of enzyme extract. The rate of the reaction is calculated from measurements of the absorption at 30° C and 290 nm taken at 10 min intervals, as a minimum an hour after the addition of phenylalanine, using a Beckman DU-5 spectrophotometer. The blank contained only buffer and enzyme extract. Incubations were carried out immmediately after preparation of a crude extract from the tissue. The molar extinction coefficient of *trans*-cinnamic acid dissolved in 0.1 M borate buffer at pH 8.8 was 9,600 at 290 nm, according to results obtained by other authors (15, 24, 34). Protein was measured by the method of LOWRY *et al.* (22) using desiccated bovin serum albumin as standard.

Results

The evolution of PAL activity during the life cycle of barley plants (*Hordeum distichon* L.) is shown in figure 1. In this

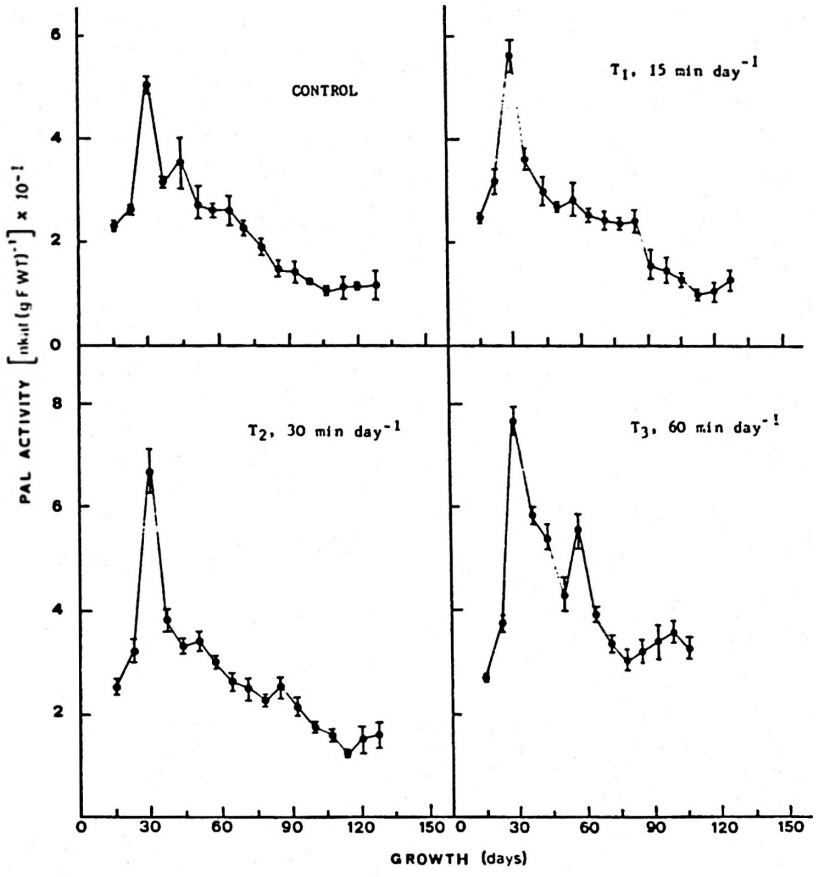

Fig. 1. *Changes in PAL activity [nkat (g f. wt.)$^{-1}$] during the growth of barley plants (*Hordeum distichon*) daily exposed to UV-A radiation.*
UV-Treatments: T_1, T_2 and T_3. Each point is the mean of three determinations, and vertical bars represent the standard error of the mean.

paper, the investigated enzyme exhibited the highest activity when it was extracted from the youngest plants. A sharp and progressive decline of the activity has been observed in extracts from older plants. In any kind of treatment it has been observed that the older the plant the less is the amount of protein extracted with buffer (table II). At the same time, a direct relationship was observed between PAL activity and exposure time to UV-A radiation (355 nm) during the life cycle of barley plants. This stimulatory effect is more evident in plants exposed to high UV-A radiation dose (treatment T_3).

PAL activity increased during early stages of growth and development in this species, which coincides with the expansion of leaves. In all the treatments the maximum level of enzyme activity was detected at 29 days after sowing. By that time, the plants had received 375, 750 and 1,500 min of UV-A radiation respectively (table I). The general shape of the curves (fig. 1) for the plants exposed to little irradiation time and for control plants was

Table I. *Exposure time to UV-A radiation and growth time for each samples.*
UV treatments: T_1 (15 min day^{-1}), T_2 (30 min day^{-1}) and T_3 (60 min day^{-1}).

Samples	Growth time (days)	Exposure time (min)		
		T_1	T_2	T_3
1	15	195	390	780
2	22	285	570	1,140
3	29	375	750	1,500
4	36	465	930	1,860
5	43	555	1,110	2,220
6	50	645	1,290	2,580
7	57	735	1,470	2,940
8	64	825	1,650	3,300
9	71	915	1,830	3,660
10	78	1,005	2,010	4,020
11	85	1,095	2,190	4,380
12	92	1,185	2,370	4,740
13	99	1,275	2,550	5,100
14	106	1,365	2,730	5,460
15	113	1,455	2,910	—
16	120	1,545	3,090	—
17	127	1,635	3,270	—

very similar. These treatments showed a similar behaviour. However, 43 days after starting the experiment, the plants exposed to high UV-dose showed more alterations in the levels of PAL activity.

The activity of PAL decreased during the development of the ear, between 82 days for control plants and 85 days for irradiated plants (only treatments T_1 and T_2) and 106 days after sowing. After the 106th day, and during the senescence of the plants (106-127 days after sowing), a slight increase in this activity was observed in the plants exposed to 15 and 30 min day^{-1}. The same effect also took place during the senescence of the plants daily exposed to high UV-dose, which never completed their ontogenic cycle. PAL activity remained practically constant during the senescence of the control plants. At the end of the experiment the plants received 1,685, 3,270 and 5,460 min of the UV-A radiation, respectively (table I).

Table II. *PAL activity (pKat mg^{-1} protein) extracted from barley plants exposed to UV radiation during its life cycle.*
UV treatments: T_1, 15 min day^{-1}; T_2, 30 min day^{-1} and T_3, 60 min day^{-1}. Each value represents the mean ± SE from three determinations.

Growth (days)	Control	T_1	T_2	T_3
15	10.7 ± 0.77	11.2 ± 0.83	11.8 ± 0.51	12.7 ± 0.26
22	12.3 ± 0.30	12.2 ± 0.35	12.5 ± 0.38	14.2 ± 0.66
29	21.5 ± 0.61	17.9 ± 0.75	20.5 ± 0.45	20.6 ± 1.00
36	17.4 ± 0.38	23.9 ± 0.65	23.8 ± 0.44	26.7 ± 0.85
43	20.0 ± 0.50	22.0 ± 0.38	23.5 ± 0.40	26.1 ± 0.45
50	17.5 ± 0.45	21.3 ± 0.68	23.3 ± 0.38	27.4 ± 0.85
57	17.0 ± 0.58	20.0 ± 0.80	22.4 ± 0.46	27.6 ± 1.39
64	15.9 ± 0.35	21.6 ± 0.53	23.1 ± 0.40	24.5 ± 0.26
71	16.0 ± 0.26	19.5 ± 0.36	22.4 ± 0.25	24.0 ± 0.39
78	15.1 ± 0.65	18.2 ± 0.66	21.8 ± 0.50	22.8 ± 1.25
85	14.2 ± 0.53	11.8 ± 0.56	20.2 ± 0.40	21.9 ± 0.80
92	13.9 ± 0.36	11.7 ± 0.26	19.7 ± 0.25	22.3 ± 0.76
99	11.0 ± 0.57	16.8 ± 0.70	19.1 ± 0.61	22.9 ± 0.80
106	10.5 ± 0.96	14.5 ± 0.56	18.6 ± 0.45	23.2 ± 0.44
113	9.5 ± 0.21	10.6 ± 0.61	17.3 ± 0.62	—
120	7.0 ± 0.51	14.8 ± 0.82	16.3 ± 0.60	—
127	7.8 ± 0.65	13.9 ± 0.69	17.6 ± 0.45	—

Discussion

Results of this paper indicate that the activity of PAL during the life cycle of barley plants has been affected by daily exposure to UV-A radiation. These results also indicate that a stimulatory effect produced depends directly upon the radiation dose received by plants. PAL activity in plants exposed to UV-A radiation was greater than in control plants (fig. 1) and this increase was more evident in plants exposed to high radiation dose (60 min day^{-1}). Daily UV-A exposure has previously been reported (4) produce an increase in PAL activity in *Ononis spinosa* plants. This effect was also directly related to the UV-dose applied to plants. The differences in PAL activity between control and irradiated plants were also in agreement with results from other authors (8, 9, 11, 27, 28, 30, 32), who clearly demonstrated a relationship between enzyme activity and exposure to UV-radiation. Irradiation, even at short wavelengths, leads to an increase activity of the enzyme PAL (12). In contrast, ANDERSEN and KASPERBAUER (1) found no significant differences in PAL activity in tobacco plants grown under visible and UV-near lights. These authors indicated that this effect might have been caused by such factors as time of sampling and the size of the harvested tobacco leaves. However, the UV responses seems to be similar in the cell cultures and in intact plants (29), but specific responses to UV-A radiation alone are unknown in higher plants.

The development of higher plants is particularly responsive to environmental factors. The relationship between PAL activity and growth time in control and irradiated barley plants might be also considered. It has been well established that the growth and the accumulation of biomass decreased in plants exposed to UV radiation (18, 25), but in some cases, stimulatory effects are obtained. Adverse effects on growth are usually accompanied by other typical stress reactions, such as irregular growth or bronzing as well as increase in phenolic compounds (flavonoids and related pigments) (2, 3, 26). At the experimental conditions used in this paper, PAL seems to be extraordinarily sensitive to the physiological state of the plants and the levels observed may be the normal ones for the growth period or they may be due to UV-radiation. The enzyme PAL showed the highest activity during early stages of growth and decreased significantly with age increase in all treatments (fig. 1). The observed decreased in PAL activity as the plant increases in size, does not appear to be caused by an inhibition of PAL activity in older plants. Consequently, the decrease in enzyme activity for each plant appears to be the result of lower enzyme levels in the older plants. This effect is more evident in plants exposed to high UV-A radiation dose, which showed an accelerated ageing.

The rapid increase in the level of PAL followed by a sudden decline, suggests a prior synthesis with a subsequent degradative process characteristic of the turnover of plant proteins. The lower levels of PAL activity in the older plants could be the result of a decrease in the synthesis or activation and/or an increase in the degradation or inactivation of the enzyme. PAL activity probably increases due to three different mechanisms of regulation, as they have been proposed: a) increase in de novo enzyme synthesis (17, 31); b) increase in enzyme activation from a pre-existing pool of inactive PAL (9, 20) and c) decrease in enzyme degradation (35). Different mechanisms of regulation have also been suggested for a subsequent decay in PAL activity: a) synthesis of a PAL-inactivating system (23); b) decrease in PAL synthesis coupled to PAL inactivation (7) and c) control of PAL activity by cinnamic acid or

further phenylpropanoid metabolites (4, 9, 16, 19).

According to LOSCHKE *et al.* (21) and the data stated above it appears that more than one mechanism may be operating simultaneously in plant tissues. The changes in PAL activity are potentially interesting in UV-A energy regulation for plant growth. However, the variations in the levels of PAL activity depend on the age and development of the plants as well as on the different organs and plant tissues. Consequently, a comparison of results concerning PAL activity in different plants or in different organs of the same plant and its relationship to development is only possible if comparable stages of growth are considered (6, 7, 13, 33). To sum up, it can be concluded that the direct effect of UV-A radiation during the life cycle of barley plants was manifested by a stimulation in the PAL activity, which was more evident at the initial stages of growth.

Resumen

Se estudia la actividad fenilalanina amonio-liasa (PAL) durante el ciclo vital de plantas de cebada (*Hordeum distichon* L.) expuestas a la radiación UV-A durante 15, 30 y 60 min día^{-1}. En comparación con las plantas control, se observa un efecto estimulante de la actividad PAL, directamente relacionado con el tiempo de exposición a la radiación. La cantidad de proteína extraída decrece significativamente con la edad de las plantas, en todos los tratamientos. El PAL muestra una alta actividad durante los primeros estadios de crecimiento y un fuerte y progresivo descenso a medida que se incrementa la edad de las plantas, más evidente durante el desarrollo de la espiga.

Palabras clave: *Hordeum distichon*, Cebada, Actividad fenilalanina amonio-liasa (PAL), Radiación UV-A.

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