Phytochemistry, 1978, Vol. 17, pp. 1545–1547. © Pergamon Press Ltd. Printed in England. 0031-9422/78/0901-1545 $02.00/0

PHOTOCONTROL OF PHENYLALANINE AMMONIA-LYASE IN BARLEY SEEDLINGS TREATED WITH PYRIDAZINONE INHIBITORS OF CHLOROPLAST DEVELOPMENT*

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(*Received* 5 *January* 1978)

Key Word Index—*Hordeum vulgare*; Gramineae; *Phaseolus aureus*; Leguminosae: Sandoz 6706; Norflurazon; plastids; PAL; blue light.

Abstract—Seedlings imbibed for 48 hr in aqueous solutions of the pre-emergent herbicide Sandoz 6706, or its presumably active conversion product Norflurazon, grow into albino plants in white light. Neither herbicide has any effect on PAL in dark grown barley shoots. In white light, however, pretreatment with 100 μM herbicide causes an increase in barley shoot PAL of about 50% over that found in untreated plants. Barley root PAL is stimulated by 0.1 μM Sandoz 6706 but inhibited by higher concentrations. Mung bean primary leaves show dose responses similar to barley roots. The herbicides have no effect in continuous red light, yet blue light is as effective as white light in eliciting PAL responses. The results are discussed in relation to the subcellular distribution of PAL.

INTRODUCTION

Brief seed treatments with the pre-emergent herbicide Sandoz 6706 [San 6706; 4-chloro-5-(dimethylamino)-2-(α,α,α-trifluro-*m*-tolyl)-3(2H)-pyridazinone] produces albinioc plants in a wide range of species [1]. San 6706 is presumably *N*-demethylated to Norflurazon before exerting its effect [2]. In the dark, herbicide-treated seedlings undergo normal etiolated development but contain virtually no carotenoids [3]. When grown in high levels of white light, the seedlings display normal photomorphogenetic development [3]. Although treated seedlings normally die when their stored metabolites are depleted, this can be largely circumvented by feeding exogenous sucrose [4].

San 6706 treatment inhibits chlorophyll accumulation and carotenoid production [5], but these effects can be reduced by concomitant applications of phytol, farnesol, or certain unsaturated fatty acids or their esters [6]. Phytoene accumulates in barley seedlings [7], *Chlorella fusca* [8], and the myxobacterium *Myxococcus flavus* [9] when treated with San 6706. It is likely that the herbicide blocks cyclization reactions in the biosynthetic pathway to those carotenoids [5] which normally prevent chlorophylls being photooxidized in high levels of white light [2, 3, 5].

San 6706 also inhibits the Hill reaction and photosynthetic CO_2 fixation in isolated chloroplasts [2]. Feierabend and Beevers [10] report that it also reduces levels of the microbody enzymes catalase and glycolate oxidase in light-grown wheat seedlings. This may due to its effect on the lipid composition of plastid membranes [6] in that photocontrolled differentiation of peroxisomes takes place only if microbodies are able to attach themselves to outer membranes of plastids [11].

At the ultrastructural level, San 6706-treated etioplasts are morphologically identical to those of control plants, both producing prolamellar bodies and ribosomes. Under white light conditions plants treated with San 6706 produce normal populations of cytoplasmic 80S ribosomes, but the plastids have no detectable 70S ribosomes [3]. Plastids from the herbicide-treated plants lack grana but contain unusually long and disorganized thylakoids [3, Blume unpublished]. San 6706 has no effect on 70S ribosomes *in vitro*; presumably, unstabilized photosensitized chlorophyll or its precursors oxidatively destroy plastid ribosomes in the light [3].

As phenylalanine ammonia-lyase (PAL) is photocontrolled in barley plastids [12], we investigated the effect of San 6706 and Norflurazon on PAL activity in barley and mung bean seedlings. We are not aware of previous studies on the effects of San 6706 on PAL, although there are reports that the herbicide increases anthocyanin levels in wheat seedlings [4].

RESULTS AND DISCUSSION

San 6706-treated barley seeds germinate and grow as well as control plants for a week or more, expressing characteristic phytochrome-controlled leaf unrolling and cessation of blade elongation. After this the seedlings wilt and die as metabolic reserves are depleted. Herbicide-treated mung bean seedlings also express normal morphogenetic changes for the first four to five days after planting but thereafter rapidly wilt and show general leaf necrosis.

Untreated six-day-old barley shoots contain 950 μmol total chlorophyll/g ft. wt when grown under continuous white light. Chlorophyll levels are reduced to 670 μmol/g fr. wt if the plants are germinated for the first two days in 0.1 μM solutions of San 6706, but only 7 μmol chlorophyll if treated with 100 μM solutions of the herbicide.

* Part 7 in the series 'Phenolic Biosynthesis in Barley Seedlings.' For Part 6 see ref. [23].

Table 1. Effects of Sandoz 6706 and Norflurazon on PAL in seedlings grown under continuous white light

Treatment	PAL activity (nmol cinnamic acid/min/g fr. wt) 4 day	5 day	6 day
		Barley shoots	
Control	86 1	76.8	62.0
San 6706	122.3	95.7	71.8
Norflurazon	111.5	99.2	74.2
		Mung bean leaves	
Control	74.2	100.8	104.6
San 6706	112.4	84.5	31.4
Norflurazon	128.5	90.3	38.7

Seedlings were germinated for two days on filter paper with 100 μM Sandoz 6706 or 200 μM Norflurazon (see Experimental) solutions and transplanted to vermiculite. The seedlings received 5.5 k-erg/cm^2/sec of continuous white fluorescent light, and were grown at 26°, throughout the experiment.

No herbicide treatments had any appreciable effect on total protein content of six-day-old seedlings.

The effects of San 6706 and Norflurazon on PAL levels in barley and mung bean seedlings grown under continuous white fluorescent light are shown in Table 1. PAL activity in barley reaches a peak several days after germination and slowly declines thereafter [13]. San 6706 enhances PAL activity in light-grown barley shoots, but does not affect PAL levels in dark-grown plants (Table 2). The rapid decrease in PAL activity of treated mung bean seedlings on the 5th and 6th day correlates with wilting and necrosis. Norflurazon gives responses essentially similar to San 6706 in either barley or mung bean. Since other workers have used San 6706 more often than Norflurazon, subsequent experiments on PAL were restricted to San 6706 effects.

To determine dose effects of San 6706 on PAL, plants were treated with various levels of the herbicide, grown under continuous white light, and harvested on the 5th day (Fig. 1). Promotion or inhibition is dose-dependent and roots are much more sensitive than are shoots.

Surprisingly, while 100 μM San 6706 has no effect on PAL in dark-grown barley shoots, this concentration inhibits PAL in the roots of either dark- or light-grown barley. PAL levels in control and San 6706-treated roots were, respectively, 175 and 123 units (nmol cinnamic acid/min/g fr. wt) for light-grown tissues and 107 and 85 units in the dark. Other than reports that San 6706 treatment blocks carotenoid formation in dark-grown tissues [14]. we are not aware of any other reported effects of San 6706 on dark-grown plants. As San 6706 apparently works within plastids, and as all higher plant cells contain plastids of some sort, the effect of this herbicide on PAL in the dark may relate to reports of PAL in proplastids [15] or etioplasts [16].

Table 2. Influence of light on PAL and chlorophyll content of barley shoots treated with Sandoz 6706

Light Treatment	PAL activity (nmol cinnamic acid/ min/g fr. wt)	Chlorophyll (μg/g fr. wt)
White, control	76.8	950
San 6706	95.7	7
Blue, control	68.4	232
San 6706	89.8	44
Red, control	62.9	207
San 6706	63.3	18
Dark, control	53.4	(none)
San 6706	54.0	(none)

Seedlings were grown as described in the legend to Table 1. Whole shoots were harvested on the 5th day after planting. White light (5.5 K-erg/cm^2/sec), blue light (620 erg/cm^2/sec), or red light (650 erg/cm^2/sec) treatments were continuous. Carotenoids were not detected in the San 6706-treated plants.

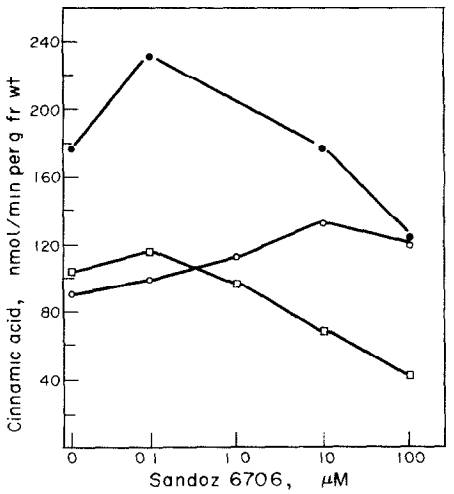

Fig. 1. Effects of increasing concentrations of San 6706 on PAL activity in 5 day old barley and mung bean seedlings. Seedlings were grown under continuous white light and treated as described in the legend to Table 1. PAL was determined in barley roots (●), barley shoots (○), and in primary leaves of mung beans (□). PAL in mung bean roots (not shown) was progressively inhibited as herbicide levels increased

The production of carotenoid precursors up to the point of phytoene is not inhibited by this herbicide [8], and exogenous sucrose supports new growth in San 6706-treated barley seedlings which are reported to contain no chlorophylls or carotenoids [4]. These studies all point to specific blocks of plastid carotenoid synthesis as the basis for San 6706 effects.

Between 20 and 30% of the PAL in photosynthetic tissues is associated with thylakoids in organisms ranging from the green alga *Dunaliella* to vascular plants such as wheat [17]. San 6706 has no effect on thylakoid formation in plants grown in darkness or light [3] and purified barley etioplasts contain about 27% of the PAL in whole shoots [12].

As a working hypothesis, we suggest that the San 6706-induced changes in lipid components of thylakoids [2] are accompanied by a change in their protein composition. This may divert protein synthesis into making more PAL or, alternatively, may activate PAL normally repressed in untreated plastids.

PAL levels in mung bean leaves were slightly promoted by San 6706 at one hundredth the concentration which gave maximal promotion in barley shoots; higher levels were inhibitory (Fig. 1). PAL in mung bean roots was progressively inhibited as San 6706 levels were increased. Thus mung beans respond to this herbicide in a fashion generally similar to barley, although with more sensitivity.

As chlorophyll bleaching is a wavelength-dependent process, and since PAL photoinduction has been studied in barley in detail [12, 13], we investigated the effects of San 6706 on PAL in barley seedlings grown under continuous blue or red light. The results are shown in Table 2. San 6706 has no effect on PAL in red light, but increases this enzyme about 25% in plants grown under either blue or white light. Chlorophyll levels are slightly higher in both control and treated plants grown under blue light than in red light. There is no apparent relationship between chlorophyll content and PAL levels in these plants.

Phytochrome control of barley leaf unrolling and expansion is not altered by San 6706, and the effect of blue light on PAL does not appear to modify any basic mechanism(s) controlled by phytochrome. Carotenoids are absent from barley shoots treated with high levels of San 6706 [4, Blume unpublished] and these may be eliminated as blue light photoreceptors in San 6706-directed effects on PAL.

Engelsma [18] has shown that blue light, mediated by a flavine photoreceptor, will cause *cis, trans* isomerization of (hydroxy) cinnamic acid(s) in *Cucumus* seedlings which correlates with increased PAL levels. Several workers have presented data in support of PAL control through a feedback system in which the *trans* isomers of hydroxycinnamic acids are considerably more inhibitory than are the *cis* isomers [16, 18]. Plastids of barley and other plants [16] accumulate cinnamic acids. In the absence of the blue-light-absorbing chlorophylls and cyclized carotenoids, one would expect considerable amounts of *cis* isomers of cinnamic acids in plants grown under blue light, perhaps mediated by plastid flavines.

San 6706 seems to be an excellent probe with which to study physiological processes in plants containing no carotenoids, and in which the degree of development of chlorophylls and 70S plastid ribosomes can be manipulated by varying herbicide or light treatments. Investigations are now underway to isolate and purify plastids from San 6706-treated plants in a further study of the subcellular distribution of phenolic enzymes and secondary phenolics. Our work on the effects of this herbicide on flavonoids is reported in the next paper in this series [19].

EXPERIMENTAL

Herbicides, plant material and light treatments. Sandoz 6706 or Norflurazon were dissolved in Me_2CO applied to 9 cm discs of filter paper, and the discs air dried. Controls were treated with Me_2CO alone. The discs were placed in petri dishes and 10 ml H_2O added to solubilize the herbicides. As Norflurazon is translocated with only one half the efficiency of Sandoz 6706 [20], it was applied at twice the concn of the latter. Atlas 68 barley [13] or commercial mung bean seeds were germinated for 48 hr on the treated filter paper discs under light conditions to be applied throughout the experiment. After 48 hr, seedlings were removed from the petri dishes, transplanted to water-satd vermiculite, and returned to the culture chambers. All plants were grown at 26°. White light grown plants received continuous cool-white fluorescent light at an intensity of 5.5 k-erg cm^2 sec. Broad-band blue and red light sources [13] provided 620 and 650 erg cm^2 sec, respectively, of continuous illumination.

Assays. The spectrophotometric assay for PAL in whole shoots of barley seedlings or primary leaves of mung beans has been previously described [21]. Chlorophyll was measured by the method of ref. [22].

Acknowledgements—Sandoz 6706 and Norflurazon was a gift of Sandoz, Inc., Homestead, Florida. The research was supported by a grant from the Miami University Research Committee.

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