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PHYTOCHROME-MEDIATED PRODUCTION OF MONOTERPENES IN THYME SEEDLINGS

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Key Word Index—*Thymus vulgaris*; Labiatae; thyme; glandular trichome; essential oil; monoterpene; thymol; biosynthesis; light; phytochrome.

Abstract—Irradiation of either etiolated seedlings or detached cotyledons of thyme with red light strongly promoted the production of monoterpenes (thymol, γ-terpinene, *p*-cymene, and carvacrol). This photoresponse proved to be reversible by alternate irradiations with red and far-red light, suggesting for the first time the participation of phytochrome in the photoregulation of monoterpene production.

INTRODUCTION

Many labiate plants including thyme (*Thymus vulgaris* L.) are characterized by the presence of glandular trichomes in the leaves. Amelunxen *et al.* [1] and Venkatachalam *et al.* [2] reported the accumulation of essential oils in the isolated glandular trichomes of peppermint and sage, respectively. We had also shown that the peltate glandular trichomes isolated from thyme cotyledons contain the monoterpenes thymol, γ-terpinene, *p*-cymene, and carvacrol [3]. Furthermore, the total amount of monoterpene in different organs of the seedlings was correlated with the number of trichomes, as was reported for the leaves of *Perilla* [4] and sage [2], suggesting that the trichome is probably the main site of monoterpene accumulation. Recently, we have found that white light promotes monoterpene accumulation in etiolated thyme seedlings. Although light is known to modify the composition and yield of monoterpenes in higher plants [5–9], its action mechanism has never been elucidated. The present experiments with thyme seedlings have been conducted to demonstrate the participation of phytochrome in photoregulation of monoterpene production and trichome formation in higher plant.

RESULTS

Cotyledons, hypocotyls and radicles separated from four-day-old etiolated seedlings were irradiated with red light (R) for 48 hr. Exposure of the excised cotyledons to R markedly stimulated the production of monoterpenoids, especially of thymol (Table 1). The four major monoterpenes detected in the seedlings were mainly accumulated in cotyledons 48 hr after irradiation, whereas no monoterpenes were detected in the excised radicles. These results indicate that cotyledons are the main site of monoterpene synthesis in thyme seedlings.

To determine whether the photostimulation of monoterpene production is mediated by phytochrome, the R/FR photoreversibility was examined. Etiolated seedlings irradiated with either R or far-red light (FR) for periods of 5, 15, 30 and 60 min were incubated in the dark for 24 hr before measuring the thymol content in consideration of a time lag for monoterpene synthesis [3]. Exposure of seedlings to R (2.0 W/cm^2) for 15 min or more resulted in a statistically significant increase over the dark control regarding thymol production in cotyledons (Fig. 1). By contrast, the seedlings irradiated with FR (2.0 W/cm^2) showed no increase in thymol produc-

Table 1. Effect of red light (3.0 W/m^2, 48 hr) on monoterpene production in detached organs of four-day-old etiolated seedlings of thyme

Organ	Treatment	Ave. fr. wt per seedling (mg)	Monoterpene (pmol/organ)			
			Thymol	Carvacrol	γ-Terpinene	*p*-Cymene
Cotyledons	Dark	1.14	232±42*	59±9	67±20	34±1
	Red light	1.27	823±60	97±5	200±12	71±9
Hypocotyl	Dark	1.46	n.d.†	n.d.	n.d.	n.d.
	Red light	1.23	17±6	18±6	trace	trace

*Mean ±s.e. of six replicates.
†Not detected (detection limit: <0.2 pmol/organ).

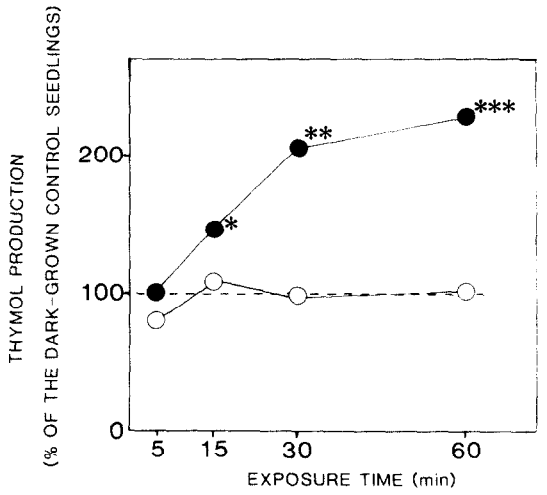

Fig. 1. Effect of red (R, 2.0 W/m^2, –●–) and far-red (FR, 2.0 W/m^2, –○–) lights on thymol production in cotyledons of 4-day-old etiolated thyme seedlings. Asterisks, *, ** and ***, indicate statistically significant differences from the dark-grown control at 5, 1 and 0.1% levels, respectively (6 replicates).

tion, compared with the dark-grown counterparts. The stimulatory effect of a short-term irradiation (30 min) with R was completely nullified by a subsequent exposure (30 min) to FR (Table 2). Such photoreversibility was observed for the formation of thymol and its biosynthetic precursor γ-terpinene even when the alternate irradiations with R and FR were repeated.

Exposure (30 min) of four-day-old etiolated seedlings to R also resulted in a significant increase in the number of trichomes per cotyledon as well as in the amount of thymol per trichome (Table 2). The stimulatory effect of R, however, was nullified by the succeeding irradiation (30 min) of seedlings with FR, suggesting that phytochrome also is involved in trichome formation.

DISCUSSION

In labiate plants, several workers [2, 4] reported that the number of trichomes was correlated with the amount of monoterpenes. The present study has also shown that monoterpene production is associated with trichome formation under the control of phytochrome. This result is in accordance with the previous demonstration of monoterpene accumulation in the isolated trichomes of thyme [3]. Thus it is considered that the photostimulation of monoterpene production is partly due to an increase in the number of newly developed trichomes after irradiation with R.

There are a number of reports that red light promotes the accumulation of various terpenoids and sterols in higher plants. In maritime pine, R stimulated the synthesis of mono- and sesquiterpene hydrocarbons [9]. Irradiation of etiolated sunflower seedlings with pulses of R led to an increased accumulation of sesquiterpene lactones, whereas its effect was partly quenched by a subsequent treatment with FR [10]. Loveys and Wareing [11] reported that a short exposure of etiolated wheat leaf to R stimulated the synthesis of gibberellin. In etiolated oat seedlings, R was especially effective on phytol accumulation [12]. Sterol accumulation was stimulated by R in dark-grown seedlings of *Digitalis purpurea*, although the exposure to R subsequent to the FR treatment failed to show a stimulatory effect [13]. It has been well established that carotenoid biosynthesis is promoted by R through phytochrome in higher plants [14, 15].

The present study has demonstrated for the first time that phytochrome mediates the light-induced increase of monoterpene production. Thus, it is suggested that the production of various terpenoids in etiolated seedlings of higher plants might be primarily stimulated by R through phytochrome.

EXPERIMENTAL

Plant material. Thyme (*Thymus vulgaris* L.) seeds were purchased from Takii Seeds Co. Ltd, Kyoto. *Ca* 240 seeds were germinated on sterile absorbent cotton moistened with 20 ml Shive's R552 medium in each 50 ml beaker as described previously [3] at 25° in the dark.

Light sources. Red light (R, 600–700 nm) was provided with red fluorescent lamps (FL 20S Re-66, Toshiba) through a red acrylic filter, Acrylite type 102 (Mitsubishi), fluence rate 2.0 or 3.0 W/m^2. Far-red light (FR, 700–800 nm) was obtained by the

Table 2. Photoreversible formation of monoterpenes and peltate glandular trichomes in 4-day-old etiolated seedlings of thyme

Treatment	Monoterpene content (pmol/two cotyledons)				No. of trichomes per 2 cotyledons	Diameter of trichomes (μm)
	Thymol	Carvacrol	γ-Terpinene	*p*-Cymene		
Dark	—	—	—	—	52.2 ± 3.8‡	30.6 ± 0.7
R	53.4 ± 6.1**†	23.6 ± 2.8	20.3 ± 3.5**	16.1 ± 0.5	65.0 ± 4.2*	31.2 ± 0.6
FR	—	—	—	—	45.8 ± 2.6	29.5 ± 0.6
R/FR*	31.7 ± 3.0	20.2 ± 2.8	10.9 ± 2.9	13.2 ± 2.2	50.8 ± 3.8	29.8 ± 0.6
R/FR/R	69.5 ± 6.0***	22.3 ± 2.1	22.1 ± 1.8**	17.5 ± 0.2	—	—
R/FR/R/FR	47.8 ± 7.7	21.0 ± 1.8	14.2 ± 1.1	16.5 ± 0.4	50.9 ± 3.0	29.4 ± 1.2
R/FR/R/FR/R	75.2 ± 9.9***	21.9 ± 2.2	26.1 ± 2.2**	18.0 ± 2.7	68.9 ± 4.7*	34.7 ± 0.9**

*Consecutive irradiations with R (2.0 W/m^2, 30 min) and FR (2.0 W/m^2, 30 min).

†Mean ± s.e. of 7 replicates. Asterisks, ** and ***, indicate statistically significant differences from R/FR at 1 and 0.1% levels, respectively.

‡ Mean ± s.e. of 10 replicates. Asterisks, * and **, indicate statistically significant differences from Dark, FR, R/FR and R/FR/R/FR at 5 and 1% levels, respectively.

combination of two filters, Acrylite type 102 and IR-1 (NEC), with FL 20S FR-74 lamps (Toshiba), fluence rate 2.0 W/m^2.

Illumination. To study monoterpene production in excised organs, each organ was separated from 4-day-old dark-grown seedlings with a pair of scissors under dim green safelight. These organs were immediately placed on a filter paper moistened with dist. H_2O and treated with R (3.0 W/m^2) for 48 hr. In photoreversibility experiments, 4-day-old etiolated seedlings were exposed to one of the following light regimes: 5, 15, 30 or 60 min of R (2.0 W/m^2); 5, 15, 30 or 60 min of FR (2.0 W/m^2); and, alternate irradiation of R (5, 15, 30 or 60 min) and FR (5, 15, 30 or 60 min). After each treatment with light, the seedlings were placed again in the dark at 25° for 24 hr, before their cotyledons were harvested for chemical analysis and microscopic observation of peltate glandular trichomes (PGT).

Chemical analysis. Monoterpenes were extracted from samples of 100 cotyledon pairs with Et_2O (0.2 ml) and analysed by using GC, as described earlier [3]. Their contents were expressed as amounts per pair of cotyledons, as the increase in the fresh weight of cotyledon owing to brief irradiation was less than 8%.

Microscopy. Seedlings were fixed in a 2% aq. soln of H_2CO. The number of PGT in 10 cotyledons was counted by using an inverted light microscope (Diaphoto-TMD, Nikon).

Statistical treatment. All data are expressed as means ± s.e. from 6–10 samples. Differences between the dark controls and the light treatments were tested for statistical significance by Student's *t* test.

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