

Time Dependence of Phytochrome-mediated Carotenoid and Chlorophyll Synthesis in *Sorghum bicolor* L.

V. K. RAJASEKHAR, SIPRA GUHA-MUKHERJEE and SUDHIR K. SOPORY†

Plant Research Laboratory, School of Life Sciences, Jawaharlal Nehru University, New Delhi-110067, India

Accepted: 1 October 1982

ABSTRACT

In 5-day-old etiolated *Sorghum* seedlings, red light irradiation for 1 s enhanced carotenoid and chlorophyll accumulation, and 5 min of red light treatment saturated the photoresponse. The degree of red/far-red photoreversibility of carotenoid accumulation was dependent on the age of the plant. No significant escape from far-red reversibility was observed up to 30 min after the onset of a saturating red light pulse in 5-day-old etiolated seedlings. Thereafter, the escape was relatively fast and completed within 180 min.

Key words: *Sorghum bicolor* L., carotenogenesis, phytochrome, time dependence, chlorophyll synthesis.

INTRODUCTION

In spite of several reports on phytochrome-mediated pigment synthesis in plants (Mohr, 1972; Smith, 1975); studies on the effect of age of the plant on these photoresponses are incomplete. Investigations have revealed that (i) the rate of protochlorophyllide regeneration following a single irradiation (Akoyunoglou and Siegelman, 1968); (ii) the lag phase, occurring during greening in normal etiolated seedlings (Sisler and Klein, 1963); (iii) the time courses of chlorophyll synthesis as well as changes in chlorophyll *a/b* ratio under intermittent and continuous illumination (Sisler and Klein, 1963; Akoyunoglou and Argyroudi-Akoyunoglou, 1969); (iv) the effect and reversibility of red and far-red light pre-treatment on chlorophyll synthesis (Sisler and Klein, 1963; Akoyunoglou, 1970); (v) the duration of dark incubation necessary to obtain the maximum effect after an inductive irradiation (Akoyunoglou, 1970); (vi) the onset of anthocyanin synthesis mediated by phytochrome (Steinitz, Drumm and Mohr, 1976) are all markedly age dependent. However, there is no detailed information on the effect of age on phytochrome-induced carotenogenesis.

Photoreversibility is dependent upon the time interval between the red and far-red irradiation (Smith, 1975) and is lost with an increase in this time gap. But there is little information on the time for escape from reversibility during carotenogenesis.

Phytochrome controls the accumulation of total carotenoids in shoot tips of etiolated seedlings of *Sorghum bicolor* (Rajasekhar *et al.*, 1981). In view of the above comments, the effect of age and time dependency on phytochrome-mediated carotenogenesis was studied in the present investigation.

* To whom correspondence should be addressed.

MATERIALS AND METHODS

General procedures

Seeds of *S. bicolor* cv. Pusa Chari-6 were obtained from the Indian Agricultural Research Institute, New Delhi. Growth and handling of the seedlings as well as extraction and determination of carotenoids and chlorophylls were described in detail earlier (Rajasekhar *et al.*, 1981). Total carotenoids and chlorophylls were measured in shoot apices 3–4 cm in length including coleoptiles and young leaves.

Red light (λ_{\max} 650 nm, $500 \mu\text{W cm}^{-2}$) and far-red light (λ_{\max} 750 nm; $140 \mu\text{W cm}^{-2}$) were obtained as reported earlier (Sharma, Sopory and Guha-Mukherjee, 1976). A Carl Zeiss spectrophotometer, Model PMQ II or a Shimadzu UV-visible recording spectrophotometer, Model UV 240 was used to record the absorbance of extracts. Unless mentioned otherwise the results presented are the mean of at least three independent experiments.

Calculation of red/far-red photoreversibility

The photoreversibility was defined as operationally complete (100 per cent) if the effect of red light immediately followed by the same duration of far-red light was equal to the effect of the single far-red light irradiation (Sawhney *et al.*, 1980). In accordance with this, a correction for the small effect of a single far-red pulse has to be made, before calculating the reversal percentage. Therefore, the percentage photoreversibility is determined by the following equation (see also Sisler and Klein, 1963).

$$\text{Percentage reversal} = \left(1 - \frac{R/\text{FR}}{R}\right) 100,$$

where

$$\begin{aligned} R &= \text{net effect of red light} \\ &= (\text{red light effect} - \text{far-red light effect}), \\ R/\text{FR} &= \text{net effect of red followed by far-red light} \\ &= (\text{effect of red followed by far-red} \\ &\quad \text{light} - \text{effect of single far-red light}). \end{aligned}$$

RESULTS AND DISCUSSION

It was shown earlier that 5 min of red light or continuous far-red light enhanced carotenoid accumulation in 5-day-old etiolated *Sorghum* seedlings during the following 24 h in darkness and further showed an increased rate of chlorophyll accumulation when the seedlings were transferred to continuous white light (Rajasekhar *et al.*, 1981).

In a set of experiments to determine the irradiation time necessary to obtain the maximum induction of carotenoid and chlorophyll accumulation, 5 min of red light was found to saturate the photoresponse presumably by converting all the phytochrome from the Pr to Pfr form (Fig. 1A). Very short-term irradiations such as 1, 2, 5, 15 and 30 s also enhanced the accumulation of carotenoids and chlorophylls in the seedlings (Fig. 1B).

A pre-treatment with 5 min of red light followed by 24 h of darkness, or continuous far-red light for 24 h, did not cause any marked change in the inductive effect on carotenoid accumulation in 3- to 6-day-old seedlings (Table 1). Complete photoreversibility was observed even in 3-day-old seedlings. But some reversibility is gradually lost with increase in age of the seedlings from 3 to 6 days. Such a trend in results was noticed even after 6 h of white light following the 24 h dark incubation period. However, a measurable effect of red light pre-treatment on chlorophyll formation was obtained in etiolated *Phaseolus vulgaris* (Red Kidney bean) leaves only when they were more than

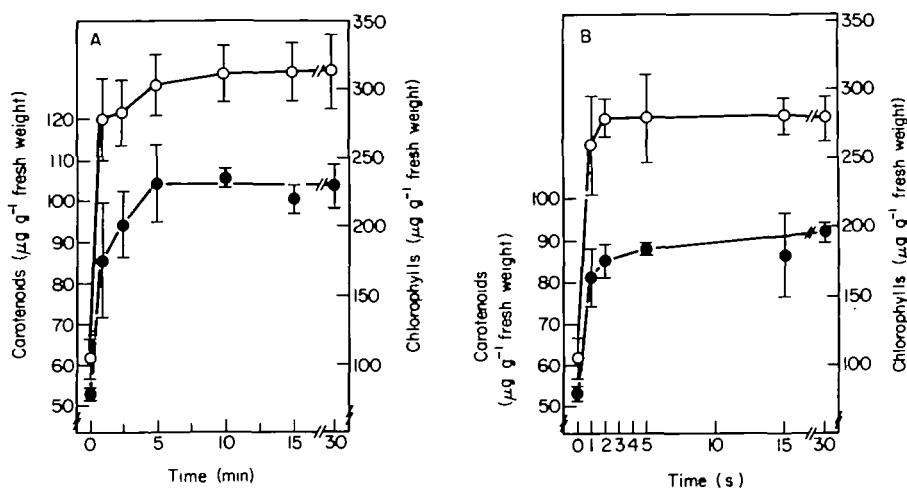


FIG. 1. Dose-response curve to increasing durations of red-light irradiation. Five-day-old etiolated seedlings were given 1–30 min (A) or 1–30 s (B) of red light and kept in darkness for another 24 h. Control set was maintained in darkness only. Following these pre-treatments, all the sets were exposed to white light (2000 lx) for a further period of 6 h, at the end of which the total carotenoids (●) and chlorophylls (○) were determined. Bars indicate \pm s.d. of escape from individual observations.

5 days old (i.e. there was no such effect in younger leaves). In addition, far-red light had no reversal effect on leaves younger than 6 days old, while on older leaves it had such an effect (Akoyunoglou, 1970). More recently, even in 60-h-old seedlings of *S. vulgare*, sensitivity to far-red light and photoreversibility of chlorophyll synthesis was demonstrated (Sawhney *et al.*, 1980). Further, in support of the present results, in the leaves of *P. vulgaris* cv. Black Valentine the red/far-red photoreversibility was hardly elicited in older seedlings (Sisler and Klein, 1963). Thus the loss of reversibility appears to increase with age. It has previously been stated that the manifestation of age dependence and specificity of photoreponse by phytochrome depends on the specific state of differentiation of a particular tissue or cell (Mohr, 1972).

In an earlier paper, it was demonstrated that photoreversibility can be achieved in phytochrome-mediated accumulation of carotenoids and chlorophylls, when the red light pulse was immediately followed by a far-red light pulse of the same duration (Rajasekhar *et al.*, 1981). In the present investigation it was noted that the reversibility can be achieved even with a considerable time gap between the inductive red light pulse and the subsequent far-red light pulse (Fig. 2). Until 30 min after the inductive red light irradiation more than 90 and 80 per cent of red/far-red photoreversibility were achieved for carotenoid and chlorophyll accumulation respectively. Although in the later period the escape from reversibility was relatively fast, the effect was completely lost only by 180 min. Similar changes in the effectiveness of photo-inactivation of red light induction by far-red light, in relation to time, have been found for hypocotyl hook opening (Klein *et al.*, 1957; Gee and Vince-Prue, 1976), leaf expansion (Downs, 1955), chlorophyll synthesis (Price and Klein, 1961; Sawhney *et al.*, 1980), ethylene production (Imaseki, Pjon and Furuya, 1971), anthocyanin synthesis (Whitelam and Johnson, 1981) and several other processes (Smith, 1975). Therefore, it would be highly desirable to consider the basis for the delayed escape from far-red photoreversibility during carotenogenesis.

As was suggested for the lag phase between red light irradiation and the start of hypocotyl hook opening in *P. mungo* (Gee and Vince-Prue, 1976), three distinct stages may be distinguished in the time period for the escape from reversibility during red-light-induced carotenoid and chlorophyll accumulation (Fig. 2). The first stage is of

TABLE 1. Effect of age of the seedlings on phytochrome-mediated carotenogenesis

Protocol of treatment		Carotenoids ($\mu\text{g g}^{-1}$ fresh weight)			
Light quality	Programme	3 days	4 days	5 days	6 days
Control	24 h dark	33 (100)	32 (100)	32 (100)	33 (100)
	24 h dark + 6 h WL	46 (100)	55 (100)	48 (100)	49 (100)
Red	24 h dark	79 (239)	77 (241)	74 (231)	76 (230)
	24 h dark + 6 h WL	108 (235)	122 (222)	103 (216)	103 (210)
Far-red	24 h dark	34 (103)	35 (103)	32 (100)	32 (97)
	24 h dark + 6 h WL	46 (100)	58 (106)	52 (108)	61 (125)
Red + far-red	24 h dark	34 (103)	38 (119)	38 (119)	44 (133)
	24 h dark + 6 h WL	49 (107)	67 (122)	69 (144)	75 (153)
Continuous far-red	24 h dark	63 (191)	85 (266)	68 (213)	69 (209)
	24 h dark + 6 h WL	82 (178)	128 (233)	100 (208)	118 (241)

WL, White light.

Etiolated seedlings of specified ages were irradiated with red and/or far-red light for 5 min of each and then brought back to darkness. The distances between shoot apices and light sources were maintained constant with the varying ages of seedlings. One set was kept under continuous far-red light, while the control set was maintained in darkness. Then, 24 h after these pre-treatments, all the sets were transferred to white light (2000 lx). Total amount of carotenoids was determined in shoot apices at 0 h and 6 h of white light treatment and expressed as $\mu\text{g g}^{-1}$ fresh weight. The relative amounts are given in parentheses.

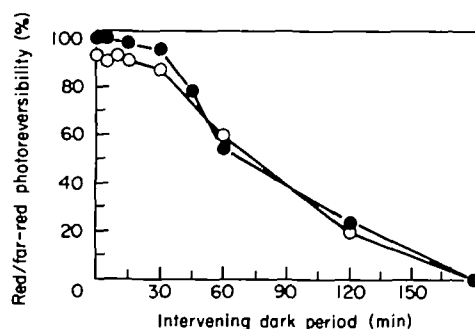


FIG. 2. Time course of escape from photoreversibility. Five-day-old etiolated seedlings were irradiated with 5 min of red light followed by far-red light pulse of the same duration. Zero to 180 min of dark intervals were maintained between the onset of red and far-red light treatments. Following the red light pulse, the seedlings were kept in darkness for a total period of 24 h and then transferred to white light (2000 lx). The sequence of treatments was then as follows: red light + dark (0–3 h) + far-red light + dark (24–21 h) + 2 h of white light. After 2 h of white light treatment the total carotenoids (●) and chlorophylls (○) were determined.

30 min duration following the red light pulse, during which almost complete photoreversibility was observed, and therefore it may be controlled by the level of the active form of phytochrome (Pfr). Sawhney *et al.* (1980) explained their data (where 100 per cent photoreversibility was achieved over a period of 40 min following an inductive red light irradiation) in terms of a delayed destruction of Pfr. After 30 min, the second stage follows

which is represented by the gradual loss of reversibility. The third stage is photo-irreversible and was observed 3 h after the red light irradiation. The time courses of these stages may vary among different species as well as among various processes (Gee and Vince-Prue, 1976; Smith, 1975). Complete reversibility to far-red is usually taken to mean that the primary effect of Pfr has not proceeded sufficiently to produce a detectable response.

It seems possible that there could be a slow and gradual phytochrome-mediated synthesis of precursors or an activation of enzymes involved in carotenoid and chlorophyll formation. Possibly, a critical level of accumulation of some factor(s) must be achieved before the manifestation of any specific effect (Gee and Vince-Prue, 1976). It is postulated that such a critical level might not have been established until the end of the first and mostly photoreversible stage. During the second, partially photoreversible stage the level of accumulated precursors, enzymes or other factors necessary for the formation of these pigments must have increased beyond the critical level and in consequence the initial processes triggered by Pfr were no longer limiting. Only then, the phytochrome-dependent changes (e.g. reconversion of Pfr to Pr) become irrelevant to the manifestation of the response (Smith, 1975) and the process is irreversible.

ACKNOWLEDGEMENTS

We thank Dr Ahluwalia, IARI, New Delhi, for the generous gift of Sorghum seeds. One of us (V. K. R.) gratefully acknowledges the award of a Senior Research Fellowship from CSIR, New Delhi. Research was partly supported by a grant from UGC.

LITERATURE CITED

- AKOYUNOGLU, G., 1970. The effect of age on the phytochrome mediated chlorophyll formation in dark grown bean leaves. *Physiologia Plantarum* **23**, 29–37.
- and ARGYROUDI-AKOYUNOGLU, J. H., 1969. Effects of intermittent and continuous light on the chlorophyll formation in etiolated plants at various ages. *Ibid.* **22**, 288–95.
- and SIEGELMAN, H. W., 1968. Protochlorophyllide resynthesis in dark grown bean leaves. *Plant Physiology* **43**, 66–8.
- DOWNS, R. J., 1955. Photoreversibility of leaf and hypocotyl elongation of dark grown red kidney bean seedlings. *Ibid.* **30**, 468–73.
- GEE, H. and VINCE-PRUE, D., 1976. Control of hypocotyl hook angle in *Phaseolus mungo* L.: the role of parts of the seedlings. *Journal of Experimental Botany* **27**, 314–23.
- IMASEKI, H., PJON, C. J. and FURUYA, M., 1971. Phytochrome action in *Oryza sativa* L. IV. Red and far-red reversible effect on the production of ethylene in excised coleoptiles. *Plant Physiology* **48**, 241–4.
- KLEIN, W. H., WITHROW, R. B., WITHROW, A. P. and ELSTAD, V., 1957. Time course of far-red inactivation of photomorphogenesis. *Science, N.Y.* **125**, 1146–7.
- MOHR, H., 1972. *Lectures on Photomorphogenesis*. Springer-Verlag, Berlin.
- PRICE, L. and KLEIN, W. H., 1961. Red, far-red response and chlorophyll synthesis. *Plant Physiology* **36**, 733–5.
- RAJASEKHAR, V. K., RAO, L. V. M., GUHA-MUKHERJEE, S. and SOPORY, S. K., 1981. Phytochrome regulation of chlorophyll and carotenoid accumulation in *Sorghum bicolor*. *Plant and Cell Physiology* **22**, 773–80.
- SAWHNEY, S., OELZE-KAROW, H., SAWHNEY, N. and MOHR, H., 1980. Control by phytochrome of chlorophyll synthesis in seedlings of *Sorghum vulgare*. *Photochemistry and Photobiology* **32**, 787–92.
- SHARMA, R., SOPORY, S. K. and GUHA-MUKHERJEE, S., 1976. Phytochrome regulation of peroxidase activity in maize. *Plant Science Letters* **6**, 69–75.
- SISLER, E. C. and KLEIN, W. H., 1963. The effect of age and various chemicals on the lag phase of chlorophyll synthesis in dark grown bean leaves. *Physiologia Plantarum* **16**, 315–22.
- SMITH, H., 1975. *Phytochrome and Photomorphogenesis*. McGraw-Hill, London.
- STEINITZ, B., DRUMM, H. and MOHR, H., 1976. The appearance of competence for phytochrome mediated anthocyanin synthesis in the cotyledons of *Sinapis alba* L. *Planta* **130**, 23–31.
- WHITELAM, G. C. and JOHNSON, C. B., 1981. Membrane permeability changes: a component of phytochrome mediated anthocyanin synthesis of *Sinapis alba*. *Phytochemistry* **26**, 9–11.

Note added in proof: Escape from photoreversibility was recently studied in chlorophyll and anthocyanin synthesis (Oelze-Karow, H. and Mohr, H., 1982. *Plant Physiology* **70**, 863–6; Dumortiez, F. M. and Vendrig, J. C., 1982. *Plant and cell Physiology* **23**, 923–7.)