

# Enhancing Effect of UV-Light on the Accumulation of Carthamine in Dyer's Saffron Florets

Koshi Saito and Yoshifumi Utsumi

Department of Bioscience and Technology, School of Engineering,  
Hokkaido Tokai University, Sapporo 005, Japan

Z. Naturforsch. **51c**, 667–670 (1996); received March 11/June 6, 1996

Dyer's Saffron (*Carthamus tinctorius*), Carthamine Accumulation, UV-Light, Enhancing Effect

UV-C (254 nm)- and UV-B (280–320 nm)-light were irradiated onto bright-yellow capitula of dyer's saffron and their effects on carthamine accumulation compared. UV-C light promotes reddening of florets more prominently than UV-B light, yielding higher amounts of carthamine after the radiation process. The enhancement of carthamine synthesis by UV-C light was investigated on cellulose columns loaded with floret extracts under O<sub>2</sub>-sufficient or O<sub>2</sub>-deficient conditions. Externally charged O<sub>2</sub> inhibits the UV-C- stimulated carthamine formation. The results are discussed in relation to light stimulation of carthamine synthesis in dyer's saffron florets.

## Introduction

Dyer's saffron (*Carthamus tinctorius* L.) includes various cultivars, of which some exhibit a colour shift to the bathochromatic side from bright-yellow to rusty red. After flowering, the transitory colour shift occurs in the floral parts, in which an orange-yellow pigment (precarthamine) is accumulated during the course of flower-head elongation (Fukushima *et al.*, 1990). The colour shift specific for the cultivar has been outlined in the literature, demonstrating that oxidative catalyses are responsible for the bathochromatic induction. O<sub>2</sub>, H<sub>2</sub>O<sub>2</sub>, KMnO<sub>4</sub>, HIO<sub>4</sub>, glucose oxidase, glycolate oxidase and amino acid oxidase are instrumental to change the colours *in vivo* and *in vitro* system (Saito *et al.*, 1983; Saito and Takahashi, 1985; Saito and Katsukura, 1992; Saito and Utsumi, 1995a,b).

The present experiments deal with photo-induction of flower colour change in dyer's saffron capitula.

## Materials and Methods

### Chemicals

Citric acid, K<sub>2</sub>CO<sub>3</sub> and acetone were purchased from Wako Pure Chemical (Osaka, Japan). Cellu-

lose powders were obtained from Funakoshi Yakuhin (Tokyo, Japan). Other chemicals and reagents used were purchased from several commercial suppliers.

### Plant material

Flowering heads from pre-flowering stage of dyer's saffron were sampled in our experimental field on early August in 1995. The outer bracteal leaves were plucked off with a pincette and bright-yellow florets laid open on the receptacles, which were used immediately for the light irradiation experiments.

### Instruments

A Toshiba GL-15 mercury lamp (254 nm, 15 W) and a Toshiba sunlamp (FL 20SE, 280–320 nm, 20W) were separately used in these studies. The spectra of these lamps are given in Fig. 1.

### Irradiation of light on flower florets (*in vivo* tests)

A light source, mercury lamp (254 nm, 15 W) or sunlamp (280–320 nm, 20 W), was held separately 27 cm (UV-C: 80 µW/cm<sup>2</sup>, UV-B: 220 µW/cm<sup>2</sup>) high above 5 flower heads, which were immersed in distilled water in a 300 ml flask and irradiated for given intervals at 24 ± 1 °C. At every time, blank runs were carried out simultaneously under

Reprint requests to Dr. K. Saito.  
Telefax: 011-571-7879.

0939–5075/96/0900–0667 \$ 06.00 © 1996 Verlag der Zeitschrift für Naturforschung. All rights reserved.

N



Dieses Werk wurde im Jahr 2013 vom Verlag Zeitschrift für Naturforschung in Zusammenarbeit mit der Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V. digitalisiert und unter folgender Lizenz veröffentlicht: Creative Commons Namensnennung-Keine Bearbeitung 3.0 Deutschland Lizenz.

Zum 01.01.2015 ist eine Anpassung der Lizenzbedingungen (Entfall der Creative Commons Lizenzbedingung „Keine Bearbeitung“) beabsichtigt, um eine Nachnutzung auch im Rahmen zukünftiger wissenschaftlicher Nutzungsformen zu ermöglichen.

This work has been digitalized and published in 2013 by Verlag Zeitschrift für Naturforschung in cooperation with the Max Planck Society for the Advancement of Science under a Creative Commons Attribution-NoDerivs 3.0 Germany License.

On 01.01.2015 it is planned to change the License Conditions (the removal of the Creative Commons License condition "no derivative works"). This is to allow reuse in the area of future scientific usage.

the same conditions as described above, except that no light was applied.

#### *Irradiation of light on glass columns (in vitro tests)*

Prior to UV-C irradiation of glass columns, 1-butanol was saturated with water. Then it was de-aerated intensively by suction with an aspirator for 10 min or charged with O<sub>2</sub> by a gas flash for 10 min at room temperature ( $23 \pm 1^\circ\text{C}$ ). Fresh florets (1 g each) were immersed in aqueous butanol (10 ml) and crushed quickly in a mortar with a pestle. The resulting slurries were filtered through a Büchner funnel. The residues were washed twice with 10 ml fresh aqueous butanol. Combined filtrates were evaporated to a small volume (1 ml) under reduced pressure below  $35^\circ\text{C}$ . The gummy condensate was loaded onto cellulose columns ( $0.75 \times 25$  cm each), which had been equilibrated with de-aerated or O<sub>2</sub> charged aqueous butanol. The columns were developed descendingly with the equilibration solvent and the column elution stopped just before the front of the butanol extracts eluted out. The extract containing cellulose columns were placed longitudinally and UV-C light (15 W) was radiated at a distance of 27 cm from the columns.

#### *Extraction of induced product*

At the end of the incubation, florets were collected and kept in boiling methanol for 10 min, then they were treated with 0.5% (w/v) K<sub>2</sub>CO<sub>3</sub>, filtered and the alkaline extracts pooled (20 ml in total). The pooled extracts were poured into a beaker, to which 0.2 g citric acid and 0.1 g cellulose powders had been added. The mixtures were treated by a magnetic stirrer for 10 min and transferred to 50 ml teflon tubes. Centrifugation was performed at 4000 x g for 5 min and the resulting supernatant discarded. Pellets were suspended in distilled water, stirred and recentrifuged (three times for 5 min, 4000 x g).

#### *Spectrophotometric measurement of induced product*

Water-washed cellulose pellets were suspended in 60% (v/v) acetone, stirred with a glass bar and transferred to teflon tubes, which were centrifuged for 5 min at 4000 x g. The acetone extraction was repeated 2–3 times through centrifugation (5 min,

4000 x g). Pooled extracts (100 ml in total) were applied for the spectrophotometric measurement of the induced product. Cellulose powders in glass columns were washed with distilled water to remove aqueous butanol and then treated with 60% acetone. The resulting acetone eluates (each 25 ml in total) were used to quantify the product yields by a Hitachi U-1100 spectrophotometer. Spectrometric readings at 521 nm were monitored for the calculation using a calibration curve.

## Results

#### *Effect of irradiation on the colour shift of florets (in vivo tests)*

Two light sources (UV-B: 20 W, UV-C: 15 W) were tested to investigate colour shift in fresh florets. The experimental data are listed in Table I.

Table I. Effect of irradiation on the formation of cathamine in florets.

Light source	Carthamine formed (pmol x min)	Increment (% of control)
Control	$1.23 \pm 0.66$	–
UV-C*	$5.70 \pm 0.45$	363.4
UV-B**	$3.75 \pm 0.39$	204.9

\* 254 nm. \*\*280–320 nm.

UV-C light accelerates positively the reddening of bright-yellow flowers. The UV-C associated colour-shift proceeds steadily and readily. At first, a red colour appears faintly on the apex region of florets, then it spreads deepening gradually towards basal parts. UV-B light also promotes the

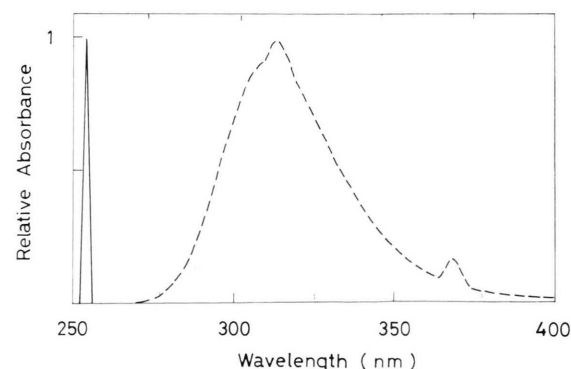


Fig. 1. Relative absorbance of UV-C and UV-B. —: UV-C, - - - - -: UV-B.

reddening process. Under the present condition, the specific enhancement of carthamine yield is estimated to be 4.6-fold in UV-C and 3.0-fold in UV-B light vs. the blank run, which received no light treatment.

#### *Time-dependent reddening of flower florets after irradiation*

Light triggers flower reddening and/or carthamine accumulation in dyer's saffron capitula. The patterns of time-dependent change in carthamine contents are illustrated in Fig. 2. UV-C acts on the

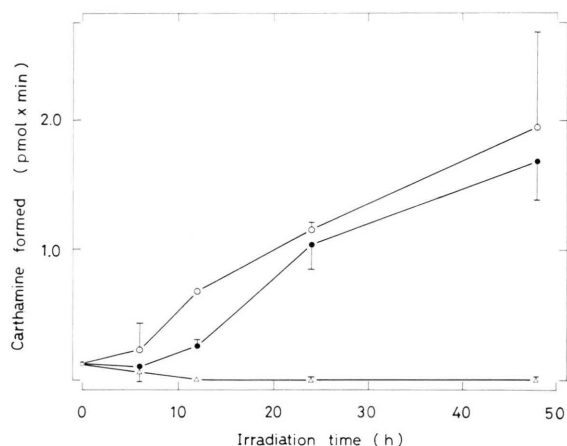


Fig. 2. Time-dependent carthamine accumulation in florets under continuous irradiation. Five flower heads were used in a series of the experiment. Data were obtained from 3–4 separate repetitions. ○ — ○: UV-C light irradiation, ● — ●: UV-B light irradiation, △ — △: blank run.

red colour appearance more effectively than UV-B light, supporting data presented in Table I. On average the velocity of carthamine accumulation is calculated to be (pmol x min): 4.26 in UV-C and 3.80 in UV-B light.

#### *Effect of oxygen on UV-induced reddening of florets*

Table II shows the effect of O<sub>2</sub> on the red colour appearance on cellulose columns under continuous radiation of UV-C light with sufficient or limiting amounts of environmental O<sub>2</sub>. Under UV-C radiation, externally charged O<sub>2</sub> inhibits carthamine accumulation: during a 3-day irradiation,

Table II. Effect of UV-C irradiation on the formation of carthamine in glass columns under O<sub>2</sub>-sufficient or O<sub>2</sub>-limiting conditions.

Condition	Carthamine formed (pmol x min)	
	A	B
Control	0.71 ± 0.05	0.46 ± 0.02
Bubbled with O <sub>2</sub>	0.67 ± 0.07	0.43 ± 0.05
Deaerated	0.69 ± 0.08	0.46 ± 0.07

A = 3-day irradiation. B = 5-day irradiation.

4.6% of carthamine is yielded. No inhibitory effect can be observed by successive UV-C radiation. By a second 3-day UV-C treatment, the carthamine yield is reduced further to 0.5%. The deaeration process also affects carthamine accumulation. An initial 3-day radiation of UV-C to a deaerated sample, reduced the carthamine content to 1.7%, while a next 3-day treatment, enhanced the red colorant formation by 2.9%. UV-C induced carthamine formation is restricted to the light-irradiated surface only.

## Discussion

Light-controlled catabolism and anabolism of metabolites have been reported in many varieties of plant materials, in which sensitized photooxidation reaction and light enhancement of biosynthesis are involved. Photochemical effects of light can occur in biological systems when more excitation energy is adsorbed by photosensitive substances via internal radiationless return to ground state; the effects include stimulation of fluorescence, delayed light emission and inhibition of the electron transport chain.

In the present study, we have investigated different efficiencies of UV-C and UV-B light on red colour development in flower florets of dyer's saffron. The experimental data are affirmative, indicating that light is a potential stimulator for floret reddening and carthamine accumulation. As light-stimulated floret reddening and red colour appearance on cellulose columns are concerned, some characteristics are evident: (1) the reaction proceeds not only in intact tissues, but in glass columns containing butanol extracts from florets, (2) red colorant is accumulated preferentially on the

surface of tissues and in the material within the glass columns which are exposed to light.

To examine the action of light on higher plants, another approach has been executed in a number of monocotyledonous and dicotyledonous species. Wellmann has discovered that UV-B (290–320 nm) has a specific effect on light-induced synthesis of flavone glucosides in cell suspension cultures of parsley, while visible light is ineffective in the reaction (Wellmann, 1971, 1974; Wellmann and Baron, 1974). In mustard seedlings, light-mediated anthocyanin synthesis seems to be exclusively under the control of the phytochrome system (Lange *et al.*, 1971; Steinitz *et al.*, 1976). These findings

may suggest that the phytochrome system participates the light-associated carthamine accumulation.

It is noteworthy that UV-C-associated formation of carthamine is decreased by externally charged O<sub>2</sub>, although floret reddening and carthamine synthesis *in vitro* are promoted by O<sub>2</sub> gas flash (Saito *et al.*, 1985a; Saito, 1993a,b). In this experiment, the mechanism by which UV-C-induced carthamine accumulation is reduced by O<sub>2</sub> has not been clarified as yet. This is the first evidence that carthamine synthesis is stimulated by UV-C irradiation. More detailed studies are in progress in our laboratory.

- Fukushima A., Takahashi Y., Ashihara H. and Saito K. (1990), Relationship between floret elongation and pigment synthesis in the florets of *Carthamus tinctorius*. *Ann. Bot.* **65**, 361–363.
- Lange H., Shropshire W. and Mohr H. (1971), An analysis of phytochrome-mediated anthocyanin synthesis. *Plant Physiol.* **47**, 649–655.
- Saito K., Takahashi Y. and Wada M. (1983), Enzymic synthesis of carthamin in safflower. *Biochim. Biophys. Acta* **756**, 217–222.
- Saito K. and Takahashi Y. (1985), Studies on the formation of carthamin in buffer solutions containing pre-carthamin and oxidizing agents. *Acta Soc. Bot. Pol.* **54**, 231–240.
- Saito K., Takahashi Y. and Wada M. (1985a), Formation of carthamin by a partially purified enzyme from safflower seedlings. *Experientia* **41**, 59–61.
- Saito K., Takahashi Y. and Wada M. (1985b), Preparation of an enzyme associated with carthamin formation in *Carthamus tinctorius* L. *Z. Naturforsch.* **40c**, 819–826.
- Saito K. and Katsukura M. (1992), The reddening of dyer's saffron florets induced by a fungal glucose oxidase. *J. Plant. Physiol.* **140**, 121–123.
- Saito K. (1993a), Glucose oxidase, a potential contributor towards flower colour modification in the capitula of *Carthamus tinctorius* L. *Biochem. Physiol. Pflanzen* **188**, 405–417.
- Saito K. (1993b), The catalytic aspects of glucose oxidase in the red colour shift of *Carthamus tinctorius* capitula. *Plant Sci.* **90**, 1–9.
- Saito K. and Utsumi Y. (1995a), Glycolate oxidase directed batho- colour change in *Carthamus tinctorius* flowers. *Biologia* **50**, 269–271.
- Saito K. and Utsumi Y. (1995b), L-Amino acid oxidase-induced transcolouration in dyer's saffron flowers. *Acta Physiol. Plant.* **17**, 37–40.
- Steinitz B., Drumn H. and Mohr H. (1976), The appearance of competence for phytochrome-mediated anthocyan synthesis in the cotyledons of *Sinapis alba* L. *Planta* **130**, 23–31.
- Wellmann E. (1971), Phytochrome-mediated flavone glycoside synthesis in cell suspension cultures of *Petroselinum hortense* after pre-irradiation with ultraviolet light. *Planta* **101**, 283–286.
- Wellmann E. and Baron D. (1974), Phytochrome control of enzyme involved in flavonoid synthesis in cell suspension cultures of parsley (*Petroselinum hortense*). *Planta* **119**, 161–164.
- Wellmann E. (1974), Regulation of flavonoid synthesis by ultraviolet light and phytochrome in cell cultures and seedlings of parsley (*Petroselinum hortense* Hoffm). *Ber. Deutsch. Bot. Ges.* **87**, 267–273.