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THE EFFECT OF KINETIN IN RELATION TO PHOTOCONTROL OF ANTHOCYANIN BIOSYNTHESIS IN *BRASSICA OLERACEA*

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Key Word Index—*Brassica oleracea*; Cruciferae; red-cabbage; anthocyanin, photocontrol, kinetin, membrane permeability, phenylalanine ammonia-lyase

Abstract—Kinetin stimulates the synthesis of anthocyanin in dark-grown seedlings of red-cabbage. When applied for only 15 min its effect resembles that of 5 min R light and can be nullified by a subsequent exposure to 5 min FR. However, kinetin fails to stimulate PAL activity in the dark-grown seedlings. It is suggested that the effect of kinetin, like that of R light, may be to increase membrane permeability, allowing a pool of endogenous substrate to reach the site of anthocyanin biosynthesis

INTRODUCTION

IT HAS been shown that kinetin promotes the biosynthesis of betacyanin in dark-grown seedlings of *Amaranthus tricolor*.[1,2] However, with the exception of work on isolated petals of *Impatiens balsamina*,[3] there appears to be little evidence that kinetin stimulates the synthesis of anthocyanin. In the present communication we provide evidence for the promotion of anthocyanin biosynthesis by kinetin in dark-grown red-cabbage seedlings.

The effects of kinetin on betacyanin and anthocyanin biosynthesis are of considerable interest because they demonstrate in an analogous way to that of kinetin on lettuce seed germination,[4] that this compound has similar effects on some morphogenetic phenomena to those of red (R) light. The investigation of the way in which kinetin acts might therefore assist in understanding how cytokinins effect morphogenetic events and at the same time might assist in the clarification of the mechanism of photocontrol of developmental processes.

In the previous studies on the influence of kinetin on betacyanin synthesis[2] and seed germination,[4] no evidence was obtained for reversal of its effects by far-red light (FR). In the case of betacyanin studies it was concluded that the kinetin stimulation of amaranthin biosynthesis appears to be unrelated to the activation of phytochrome.[2] However, a limitation in these previous investigations is that the plant materials were exposed to kinetin for periods of several hours. It is therefore very doubtful whether any evidence for a mutually reversible relationship with FR could be expected to emerge under these conditions.

[1] STOBBART, A K, PINFIELD, N J. and KINSMAN, L T (1970) *Planta* **94**, 152
[2] PIATELLI, M, GIUDICI DE NICOLA, M and CASTROGIOVANNI, V (1971) *Phytochemistry* **10**, 289
[3] KLEIN, A O and HAGEN, C W (1961) *Plant Physiol* **36**, 1
[4] MILLER, C O (1956) *Plant Physiol* **31**, 318

In the present work a 15 min high concentration kinetin treatment has been employed and the data demonstrate that a mutually reversible relationship with a short exposure to FR does exist Following our previous report concerning the low-energy phytochrome control of membrane permeability,[5] we interpret the present data to imply that kinetin, like R light, raises membrane permeability

RESULTS

A short exposure of 2-day-old dark-grown seedlings of red-cabbage to kinetin leads to an increase in anthocyanin content during a subsequent 36 hr period of dark incubation (Fig. 1) The concentration of kinetin employed (200 mg/l) was unusually high but the

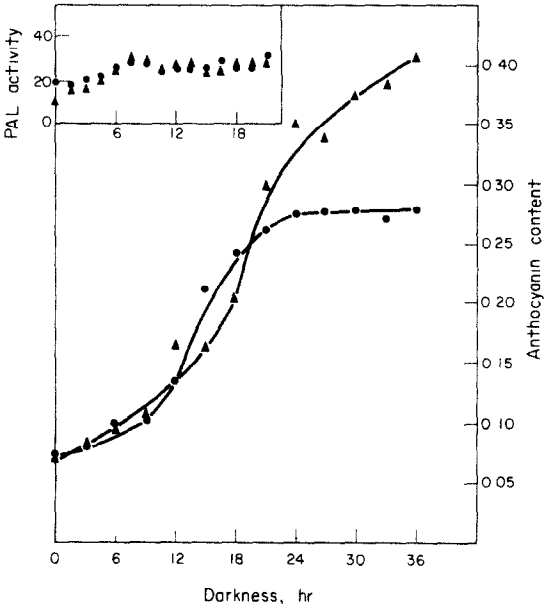

FIG 1 INFLUENCE OF KINETIN ON ANTHOCYANIN SYNTHESIS AND PAL ACTIVITY IN RED-CABBAGE 2-day-old dark-grown seedlings were treated for 15 min with 200 mg/l kinetin (▲) or H_2O (●) Changes in PAL and anthocyanin level were followed during subsequent dark incubation The PAL data are means of at least two observations and those of anthocyanin are means of 5 PAL activity is expressed as nmoles cinnamate/hr mg protein Anthocyanin is $A_{535_{nm}}$ for 1 seedling per ml in a 1 cm cell

period of treatment was only 15 min and no abnormal growth was observed to result Unlike the effect of 5 min R light, which increases the rate of anthocyanin synthesis,[5] kinetin prolongs the period of synthesis without increasing its rate (Fig 1) No increase in the activity of phenylalanine ammonia-lyase (PAL) (E C. 4 3 1.5) is evident following kinetin treatment (Fig 1)

When kinetin is applied briefly to dark-grown seedlings in conjunction with a brief exposure to R light, more pigment is formed than when it is applied in the absence of light (Table 1) If the kinetin is applied before a FR exposure, the effect of kinetin is nullified By contrast if kinetin is applied after FR, as much anthocyanin is formed as with kinetin alone This indicates that the seedlings respond to the ultimate stimulus which they receive

[5] PECKET R C and HATHOUT BASSIM T A *Phytochemistry* (in Press)

It appears that kinetin simulates the effect of R light in promoting anthocyanin synthesis and its influence, like that of R,[5] is nullified by FR. This suggests that kinetin affects the phytochrome control of biosynthesis. However, kinetin on its own is not quite as effective as R and the combination of R and kinetin produces a larger stimulation of pigment formation than either treatment on its own.

TABLE 1 EFFECT OF KINETIN ON PHYTOCHROME CONTROLLED ANTHOCYANIN SYNTHESIS IN RED-CABBAGE

Treatment*	Anthocyanin content†				
	Dark	R	FR	R/FR	FR/R
Water	0·39 ± 0·01	0·57 ± 0·03	0·40 ± 0·03	0·41 ± 0·01	0·57 ± 0·03
Kinetin before light	0·50 ± 0·03	0·63 ± 0·04	0·41 ± 0·02	0·42 ± 0·05	0·65 ± 0·04
Kinetin after light	0·50 ± 0·03	0·61 ± 0·05	0·49 ± 0·04	0·48 ± 0·05	0·62 ± 0·04

* Seedlings were grown for 2 days in darkness and then treated with either 200 mg l kinetin or H_2O for 15 min before or after exposure to R and/or FR. Period of dark incubation subsequent to treatment was 48 hr. The figures are means of 8 observations and S.E. are shown.

† $A_{525\,nm}$ for 1 seedling per ml in 1 cm cell.

We have previously presented data which suggest that the site of phytochrome control of anthocyanin biosynthesis is in a membrane which separates the enzymes involved in its synthesis from a source(s) of substrate.[5] The data presented above are interpreted to imply that kinetin, like R light, may increase the permeability of this membrane to the substrates of flavonoid biosynthesis. If this is so, it might be expected that feeding substrates to dark-grown seedlings in the presence of kinetin would increase the quantity of anthocyanin synthesized beyond the level which results from the influence of kinetin on its own. Indeed shikimic and cinnamic acids have very little effect upon anthocyanin synthesis on their own but when applied after a short kinetin treatment they promote a considerably greater production of pigment than is found with kinetin alone (Table 2).

TABLE 2 EFFECT OF EXOGENOUSLY APPLIED SHIKIMIC ACID AND CINNAMIC ACID ON ANTHOCYANIN SYNTHESIS IN RED-CABBAGE IN PRESENCE AND ABSENCE OF KINETIN

Treatment*	Anthocyanin content†
Water	0·38
Shikimic acid	0·38
Cinnamic acid	0·45
Kinetin	0·51
Kinetin + shikimic acid	0·59
Kinetin + cinnamic acid	0·61

* Seedlings were grown for 2 days in darkness and then treated with H_2O or kinetin (200 mg/l) for 15 min before transfer to the substrate solns in which they were left for a further 2 days in darkness. Concentration of shikimic acid was 50 mg/l and that of cinnamic acid was 200 mg/l. The figures are means of four observations.

† See Table 1.

DISCUSSION

Although anthocyanins and betacyanins differ from each other substantially structurally[6] and biosynthetically, recent observations demonstrate similar high sensitivities to light in the phytochrome mediated control of their production [5,7] It is evident from the data presented above that a further similarity exists with respect to the influence of kinetin [1,2]

Under conditions of short-term treatment, the stimulation of anthocyanin production by kinetin is nullified by FR It therefore appears that the effect of kinetin is similar to that of R light We have recently presented evidence to suggest that the phytochrome control of anthocyanin production involves the regulation of access of substrate through a membrane to the enzymes of flavonoid biosynthesis.[5] The data reported here are consistent with the view that kinetin too can influence anthocyanin biosynthesis by increasing the permeability of the membrane to substrates. This suggestion regarding kinetin action appears to be a new one, though a similar notion has been put forward to explain the kinetin promoted recovery of onion epidermal cells from plasmolysis [8,9]

By contrast with the nullification of the effect of kinetin by FR reported here, other workers[2,10] found no evidence of this nature in relation to betacyanin synthesis. However, it is important to note that these workers do not appear to have applied kinetin for less than 3 hr[2,10] and the failure to reverse its effect by a brief period of FR might be a function of the longer period of kinetin treatment employed in their investigations. Similarly Miller[4] was unable to demonstrate a reduction of kinetin promoted germination of lettuce by short periods of FR but the seeds were evidently kept in kinetin during and after the FR treatment.

It has been argued in a previous communication that the limitation to the production of anthocyanin in dark-grown red-cabbage seedlings[5] is not the level of PAL activity but substrate availability at the site of synthesis. The fact that kinetin promotion of dark synthesis is not preceded by any increase in PAL activity is consistent with this view

By contrast with the effects of kinetin reported here, preliminary work indicates that short-term application of gibberellic acid and abscisic acid does not lead to increased anthocyanin production [11] These findings agree with those of other workers for betacyanin synthesis [1] Indole acetic-acid has only a small promotive effect [11]

EXPERIMENTAL

Plant materials and growth conditions Seeds of red-cabbage (*Brassica oleracea* L c v Stockleys' Giant Red) were obtained from Hurst, Gunson, Cooper and Taber Ltd The growing conditions, light sources and treatments were as previously reported [5]

Anthocyanin extraction and assay As reported previously [5] The absorbance values quoted are means for samples of 10 seedlings extracted in 10 ml of 1% HCl examined in a 1 cm cell

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[11] HATHOUT BASSIM, T A (Unpublished results)

Enzyme extraction and assay As reported previously [5] The PAL values are means based upon samples of 25 seedlings extracted in 3 ml borate buffer After passing through a column, 0 5 ml aliquots were incubated in a total volume of reaction mixture of 2·5 ml

Kinetin treatment 2-day-old dark-grown seedlings were transferred in a dark room to a fresh petri dish containing 4 ml of kinetin solution followed by return to the original dish Control seedlings were treated in the same way using a petri dish of distilled H_2O

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