Temperature-dependent Expression of Betacyanin Synthesis in 
Amaranthus Seedlings

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ABSTRACT
Two phenomena related to temperature effects have been observed during the induction of betacyanin synthesis by a cytokinin (benzyladenine) in Amaranthus tricolor seedlings. One is a total inhibition of betacyanin accumulation at a temperature (39 C) at which seedling growth is unimpaired, and where there is still adequate uptake of benzyladenine. The other is the apparent induction of a higher potential for subsequent betacyanin synthesis following pretreatment of the seedlings at an elevated temperature.

The temperature dependence profile of cytokinin-dependent betacyanin induction in Amaranthus seedlings has been reported briefly (3). Further experiments detailed in the present paper investigate the relationship of this profile to growth and to benzyladenine uptake and metabolism.

Another temperature effect observed is an apparent induction of betacyanin synthesis by pretreatment of whole seedlings at 39 C, before induction at 25 C. It has already been noted that aging induction or adaptive aging of cotyledon explants is more effective following germination at 37 C rather than at 25 C (4) and it is now reported that exposure of cut seedlings to an elevated temperature (39 C) during adaptive aging further enhances subsequent betacyanin synthesis.

MATERIALS AND METHODS
Growing conditions for Amaranthus tricolor seedlings, light sources, and measurement of pigment accumulated were as previously reported (5), except that germination in the dark was at 25 C for 90 hr for some experiments. The incubation medium was usually 10 mm Na2HPO4-KH2PO4 (pH 6.3) containing 5 mm tyrosine, although in later experiments the optimum pH of 6.8 was used (6). The methods for measuring uptake and metabolism of labeled 6-[G-3H]benzyladenine have been described elsewhere (5).

RESULTS
Figure 1 gives a temperature profile for betacyanin production showing a plateau of high benzyladenine-dependent induction between 30 and 34 C. The sharp decline at temperatures >37 C is not due to a general inhibition of cell processes as shown by the data in Table I. Growth is seen to occur at temperatures where induction is inhibited (37.5 C). Conversely, induction can occur where there is little growth (<25 C). As well as growth, the uptake of benzyladenine is also much less affected by high temperature than is betacyanin induction. Figure 2 shows that there is still considerable uptake at 39 C (78% from 0.11 mm; 40% from 1.3 μm). At 35.5 C, where betacyanin accumulation is already reduced, there is no reduction in benzyladenine uptake. The metabolism of benzyladenine is altered by temperature. At both high (39 C) and low (<24 C) temperatures, the percentage of labeled benzyladenine which is converted to 7- and 9-glucosylbenzyladenine is diminished, although not as drastically as betacyanin accumulation at these temperatures.

Table II shows the preliminary induction for 4 hr at an inhibitory temperature does not prevent subsequent induction at a permissive temperature (25 C), that is, the inhibition is not due to
irreversible changes in some component of the betacyanin synthesis chain. The presence of benzyladenine during the high temperature treatment results in a somewhat higher endogenous background, but has no significant \((P > 0.1)\) effect on benzyladenine-dependent accumulation at the end of the 24-hr induction.

Although an elevated temperature during the induction period is inhibitory, pretreatment of whole seedlings at 39 C causes a stimulation of betacyanin synthesis during subsequent induction (Table III). This temperature induction can also be achieved when cut seedlings are washed in distilled H\(_2\)O during pretreatment (Table IV), rather than the germination of intact seedlings being continued at 39 C (Table III). It may be noted here that the increase observed between 2- and 3-hr pretreatments is due to the "aging" process already reported (4) and the temperature effect is thus superimposed on the aging induction.

**DISCUSSION**

The total inhibition of betacyanin synthesis at 39 C at which seedling growth is unaffected, and at which there is still considerable benzyladenine uptake, indicates that this inhibition is not due to a general inactivation of cell processes. It is possible that the temperature effect is mediated by changes in membranes and that cytokinin induction is a membrane-associated phenomenon, as already proposed on other evidence (3, 6).

The effect of temperature on benzyladenine metabolism may be instructive in this respect. The decrease in glucosylbenzyladenine formation at high and low temperatures is the reverse of what would be expected if it were the result of a lower benzyladenine degradation.
Temperature-Sensitive Cytokinin Action


Enine concentration being reached in the seedlings (cf. the lower per cent formation with 0.11 mm as compared to 1.3 μm benzyladenine) (Fig. 2). This same inverse relationship has also been reported over a wider range (5). There is a decrease in glucosyl-benzyladenine formation at high and low temperatures in a way that is not related to the total amount of benzyladenine taken up and must be related to the activity of the enzyme involved (glucosyl transferase). Glucosyl transferases are usually found in membrane fractions (1, 2). Thus, we have a correlation between inhibition of betacyanin induction and inhibition of glucosyl derivative formation at 39 C, and a possible connection between the two phenomena residing in membrane changes in response to temperature.

The temperature induction effect, or the development of a higher subsequent pigment synthesis following pretreatment at an elevated temperature, has elements in common with the water stress induction already described (5). In both cases a potential is developed during the stress pretreatment (whether it be water stress or elevated temperature) which results in increased betacyanin synthesis following relief from stress. Betacyanin can not be synthesized if stress conditions are maintained, e.g. in 0.4 M mannitol or at 39 C, but once relieved, these pretreatments result in elevated synthesis. A third analogous situation may be wounding stress (4), where there may be a wound-induced burst of ethylene involved in subsequent induction of betacyanin synthesis, but the maintenance of high ethylene during induction is inhibitory. There may thus be a common factor induced by stress which upon the release of stress allows the after-effect of increased betacyanin synthesis.

Although these environmental conditions have been considered in the present series of papers as factors influencing induction of betacyanin synthesis and therefore of importance in using this system as a cytokinin bioassay, they clearly should be considered as potential modulators in any work on ion transport or enzyme measurements using small seedlings, or explants of their roots or shoots.

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LITERATURE CITED

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