The Hormonal Control of Amaranthin Synthesis in *Amaranthus caudatus* Seedlings *


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**Summary.** Exogenous gibberellic acid, $A_3$ (GA$_3$) inhibits phytochrome mediated betacyanin synthesis in seedlings of *Amaranthus caudatus*. The growth retardants, $\beta$-chlorehyltrimethylammonium chloride (CCC), 'isopropyl-4'-(triethylammonium chloride)-5'-methylphenyl piperidine carboxylate (AMO 1618) and tributyl-2,4-dichlorobenzylphosphonium chloride (phosphon D) enhance pigment synthesis. Retardant stimulation of pigment synthesis is overcome by GA$_3$ application. Besides lowering endogenous GA levels the retardants inhibit protein synthesis by as much as 25%. Retardant inhibition of protein synthesis is not overcome by GA$_3$. The results suggest that amaranthin synthesis in *Amaranthus caudatus* can be directly controlled by endogenous GA. GA$_3$ has no effect on kinin induced dark pigment synthesis. Kinins, however, do not overcome GA$_3$ inhibition of pigment synthesis in the light.

**Introduction**

Exogenous gibberellic acid (GA$_3$) inhibits the light induced synthesis of the betacyanin, amaranthin, in *Amaranthus* seedlings (Stobart et al., 1970). The inhibitory phenomenon has enabled the development of a sensitive and rapid bioassay for gibberellins (Kinsman et al., 1975). The inhibitory responses described in these reports are to exogenous GA and hence no real conclusions can be made about the control of pigment synthesis by endogenous hormone levels. The present report describes the effects of experimentally induced low GA levels on amaranthin synthesis in seedlings of *Amaranthus caudatus*.

**Materials and Methods**

*A. caudatus* seeds were purchased from Thompson and Morgan Limited, Ipswich, England. CCC was provided by Koch and Light Limited, AMO 1618 by Calbiochem Limited, and Phosphon D by Perifleur Limited, Sussex. Radioactive amino acids were purchased from the Radiochemical Centre, Amersham.

**Growth and Pigment Extraction.** 0.2 g seeds were sprinkled on Whatman 1 filter discs in 5 cm petridishes containing 1.5 ml distilled water. After 2 or 3 days dark germination at 25° the test solutions were added and the seedlings given a 24 h illumination (white fluorescent light giving 6500 lux at seedling level) and harvested. Pigment was extracted in a small volume distilled water in a pestle and mortar. For every 4 ml H$_2$O used 1 ml 25% (w/v) trichloroacetic acid was added. After centrifugation the supernatant was removed and its A at 537 nm

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*Abbreviations.* AMO 1618, 2, 'isopropyl-4'-(triethylammonium chloride)-5'-methylphenyl piperidine carboxylate; CCC, $\beta$-chloroethyltrimethylammonium chloride; GA$_3$, Gibberellic acid, A$_3$; Phosphon D, tributyl-2,4-dichlorobenzylphosphonium chloride.

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measured. Amaranthin concentrations were calculated using a molar extinction coefficient of $5.66 \times 10^4$ (Piatelli et al., 1969a).

**GA Extraction and Bioassay.** Gibberellins were extracted and chromatographed by the methods of Stoddart (1968). The lettuce hypocotyl bioassay was carried out as described by Frankland and Wareing (1967).

**Protein Synthesis** (based on Mans and Novelli, 1961). Samples of 1 g tissue in 10 ml 0.1 M phosphate buffer, pH 7.2 were incubated with 1 µCi leucine (L-leucine—H$_3$G), 100 m—150 mCi-mmol) for 30 min and 1 h.

Linear incorporation into protein was observed up to 2 h incubation. At the end of the incubation period the ambient fluid was removed and the tissue washed $3 \times$ with a 0.1% (w/v) solution of unlabelled amino acid. The tissue was homogenised in distilled water and centrifuged $3000 \times g$, 5 min. The supernatant was made up to 10 ml and aliquots taken for protein estimations and radioactivity measurements. Protein was precipitated by the addition of 25% TCA (1 ml 25% TCA/0.8 ml aliquot + 4 ml DW). After heating at 95° for 10 min the mixtures were centrifuged $3000 \times g$ for 5 min. The precipitate was resuspended in 3 ml 5% TCA and filtered on a 2.1 cm Whatman GF/C disc in a millipore apparatus. The disc + ppt. was washed with 3 ml 5% TCA followed by 15 ml volumes of MeOH/diethyl-ether mixtures (3:1, 1:1 and 1:3, vol : vol respectively) and finally with 15 ml 1% acetic acid. The discs were dried in vials at 90° for 60 min, 2.5 ml toluene based scintillant was added [(5-phenyl oxazolyl)-benzene in 1 l toluene] and radioactivity measured in a liquid scintillation counter.

Protein was measured by the method of Lowry et al. (1951) with bovine serum albumin as standard.

**Results**

Table 1 shows the effect of GA$_3$ concentration on pigment levels in seedlings of the 1972 seed batch. A concentration of near 10 ppm GA$_3$ brings about 50% pigment inhibition. A number of growth retardants known to inhibit GA synthesis were tested for their effects on pigment synthesis. Phosphon D, AMO 1618 and CCC all promoted pigment levels by as much as 40% (Table 2). ALAR (B995), however, had little effect at low concentrations and proved inhibitory at high concentrations. In a second series of experiments growth retardants were tested at optimum concentrations in combination with 1 ppm GA$_3$. The results (Table 3) clearly demonstrate that the promotory effect of the growth retardants could be overcome by GA$_3$. In fact, pigment levels in retardant plus GA$_3$ treated seedlings were similar to levels in seedlings treated with GA$_3$ alone.

The retardants used in these experiments may block GA synthesis by preventing the formation of kaurene (Lang, 1970). Other sites of action, however, exist (Berry and Smith, 1970; Shewry et al., 1971; Heatherbell et al., 1966). To investigate these possibilities the levels of gibberellin-like compounds were determined in seedlings treated with growth retardants. The ability of the seedlings to synthesise

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<th>GA$_3$ conc. (µg/ml)</th>
<th>% pigment inhibition</th>
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<tbody>
<tr>
<td>10</td>
<td>53</td>
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<tr>
<td>1.0</td>
<td>43</td>
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<tr>
<td>0.1</td>
<td>30</td>
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<tr>
<td>0.01</td>
<td>8</td>
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Seeds were germinated in the dark at 25° for 2 days. The GA$_3$ solutions were added and the seedlings exposed to light for 24 h after which amaranthin was extracted and assayed.