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INHIBITION OF GROWTH AND ACCUMULATION OF β-CAROTENE IN CARROT ROOTS BY GIBBERELLIC ACID

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Abstract—When applied to the buds of a red variety of carrots (*Daucus carota*, var. "Rouge longue géante de Flakée"), gibberellic acid promotes the growth of the leaves and stems and inhibits that of the roots. The accumulation of β-carotene in the roots is also inhibited, while a product (or products) having strong absorption bands in the uv, with a maximum near 330 mμ, accumulates. The lutein content of the carrot roots is not greatly changed by the treatment.

INTRODUCTION

THE effects of gibberellins on growth and development of plants have been abundantly studied during the last few years. The literature shows that the observed effects are related to actions on some important metabolic processes including respiration,[1] transport of mineral nutrients,[2] accumulation of different products in the leaves,[3-6] including chlorophyll,[7] and so on. Stowe[8] and Obreiter have shown that the action of gibberellic acid on the growth of pea stem sections can be enhanced by the addition of several lipids, including the fat-soluble vitamins K_1 and E. In this case, the promotion of growth by gibberellic acid could be related to the activity of the mitochondrial fraction of the cells.

It is also well known that the gibberellins can inhibit the growth of roots of several plants.[4,9] We have shown that this is true for carrot roots, and that the effect is coupled to an inhibition of the accumulation of β-carotene by a red variety of carrot.

RESULTS

The effect of gibberellic acid (GA_3) on the growth of roots and foliage of the carrot

The effect of GA_3 on the growth of the root is very striking (Table 1). It can be seen that whereas the average weight of the control roots is circa 20 g, the foliage being a little heavier (30–40 g); when more than 200 p.p.m. GA_3 are applied, the average weight of the treated roots is very low (2–5 g per carrot), the average weight of the foliage being between 10 and 20 times heavier.

The effect of gibberellic acid on the anatomical structure and the carotene content of the roots

As can be seen in Fig. 1, the anatomical structure of the roots is strongly influenced by GA_3. In general, the cells of the treated roots are much smaller than those of the control. The amount of external parenchyma (E) is reduced in the treated roots when compared

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TABLE 1. EFFECTS OF GIBBERELLIC ACID (GA_3) ON THE GROWTH OF THE FOLIAGE AND OF THE ROOT OF CARROTS

Amount of GA_3 added p.p.m.	Fresh Weight (g)		Approximate ratio Foliage : root
	Foliage	Root	
0	35·5 (13·0–51·5)*	23·9 (25·0–49·1)*	1·5
50	40·6 (15·0–70·7)	48·0 (11·0–59·0)	1
150	59·4 (27·5–110·0)	17·9 (1·1–50·2)	3
200	71·9 (32·3–124·8)	5·6 (1·9–11·9)	13
250	40·0 (30·1–56·0)	2·0 (1·3–2·8)	20

* Maxima and minima found in the series of 15 plants.

to the amount of wood and internal parenchyma (I). The xylem strands in the control are readily defined whereas they are much more diffuse in the treated roots (Fig. 1, C and D).

The treated roots are not only smaller than those of the control; they also lose their red colour and appear either pale yellow or white. This is partly due to the presence of a relatively large zone of white parenchymatous tissues (external white zone F) near the cortex of the treated roots. This zone is almost absent from the control roots, or is very thin and restricted to the epidermis. Moreover, in the control roots, the external parenchyma under the epidermis is red, while it appears yellow or only slightly red in the treated roots. Under the microscope, the external parenchyma of the control is seen to be full of well-formed red carotene crystals, which are lacking in the external parenchyma of the treated roots (Fig. 1, A and B).

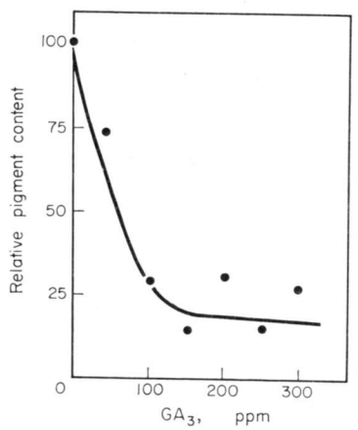

FIG. 2. EFFECT OF GA_3 ON THE RELATIVE PIGMENT CONTENT OF CARROT ROOTS.

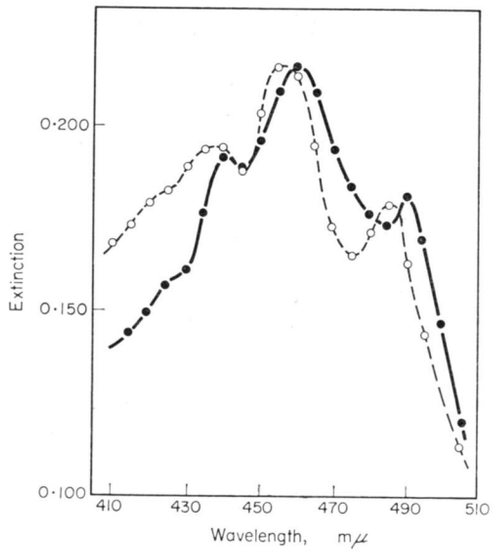

FIG. 3. SPECTRA OF CHLOROFORM EXTRACTS OF CONTROL (●———●) AND TREATED (○– – –○) CARROT ROOTS. TREATED CARROTS RECEIVED 200 P.P.M. GA_3.

The relative quantities of pigments (calculated as β-carotene) found in the roots is shown in Fig. 2. Up to 100 p.p.m. GA_3, the pigment content decreases proportionally to the applied dose of gibberellic acid. At this concentration, the roots contain about one

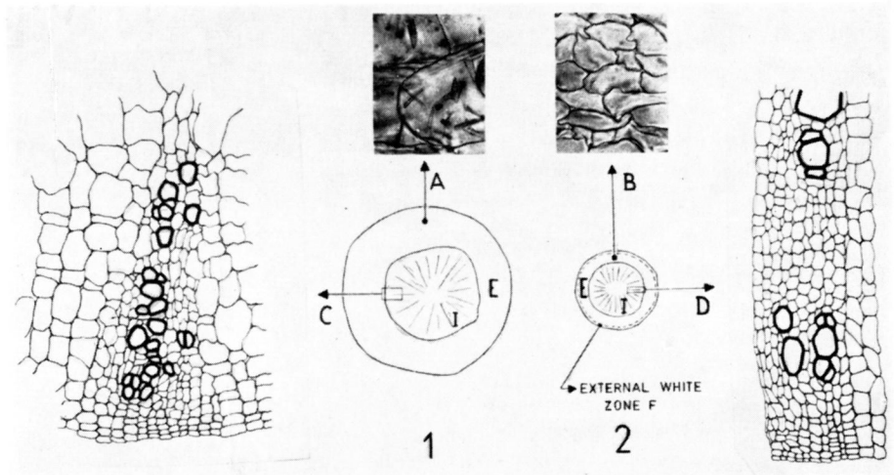

FIG. 1. STRUCTURE OF TREATED (200 P.P.M. GA_3) AND CONTROL CARROT ROOTS. 1 = control; 2 = treated roots (other explanations in the text).

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quarter of the pigments present in the control. An increase of the dose above 100 p.p.m. however does not cause any further decrease in pigment content.

The spectra of chloroform extracts of control and treated roots (200 p.p.m. GA_3) adjusted to give the same absorption value at 455–460 mμ, is shown in Fig. 3. It appears that the spectrum of the treated choloroform extract differs from the control extract in that the absorption peaks of the treated extract are noticeably shifted towards shorter wave lengths and that between 400 and 440 mμ, the absorption of the treated extracts is stronger than that of the control. This suggests that qualitative differences exist in the pigment composition of the treated and the control roots.

The composition of the extracts of control and treated roots

The elution curves obtained after chromatography of the control and the treated petroleum ether extracts (above 100 p.p.m. GA_3) on a cellulose column, is shown in Fig. 4. The control extract shows essentially three distinct peaks A, B and C (two very weak peaks B_1 and B_2 are also found in the control extract). In the treated extract, the first fraction A is lacking, or almost so; the second fraction B is present in similar amounts to that found in the control; and the last fraction C (eluted only with pure acetone) is much larger than that in the control.

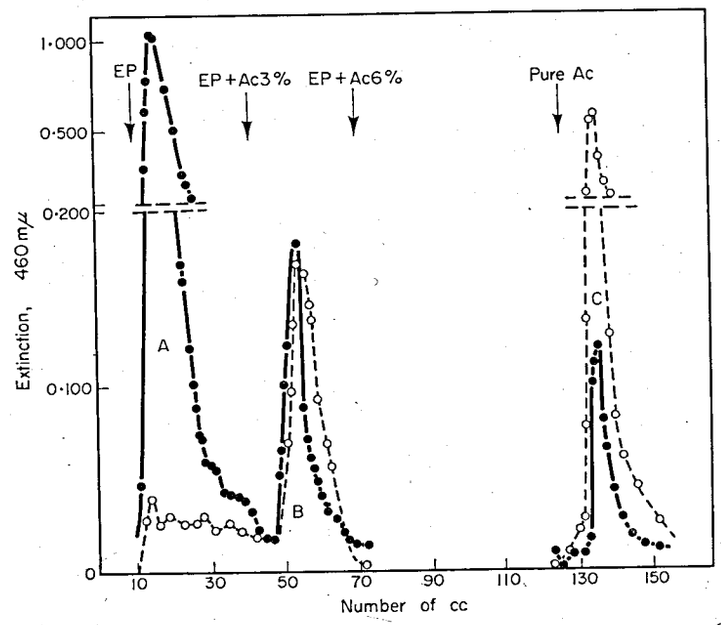

FIG. 4. ELUTION CURVES OF THE CHROMATOGRAPHY ON A CELLULOSE COLUMN FOR CONTROL (●——●) AND TREATED (○– – –○) PETROLEUM ETHER EXTRACTS (ABOVE 100 P.P.M. GA_3).

The identification of the pigments shows that fraction A consists essentially of β-carotene and that fraction B is probably lutein (although the spectra of B and its chromatographic behaviour is strong evidence for it being lutein, co-chromatography should be carried out before it can be given as definite). Fraction C contains an unknown material strongly absorbing in the UV (with a maximum near 330 mμ), probably accompanied by traces of lutein. Spectra of B and C are given in Fig. 5. We can conclude that, as a result of the action of gibberellic acid: (1) the accumulation of β-carotene is inhibited in the treated

roots, while a constituent (or constituents), the nature of which remains to be elucidated, accumulates; (2) though disturbing the accumulation of β-carotene, gibberellic acid does not greatly modify the lutein content of the roots. These two circumstances interpret the modifications induced by GA_3 in the spectrum of the rough chloroform extract (Fig. 3). The shift of the peaks is due to the disappearance of β-carotene as the major pigment, and the maintenance of lutein, while the stronger absorption of the treated extract under 440 mμ corresponds to an increased amount of a product (or products) principally absorbing in the UV. which are found in fraction C of the cellulose column chromatography.

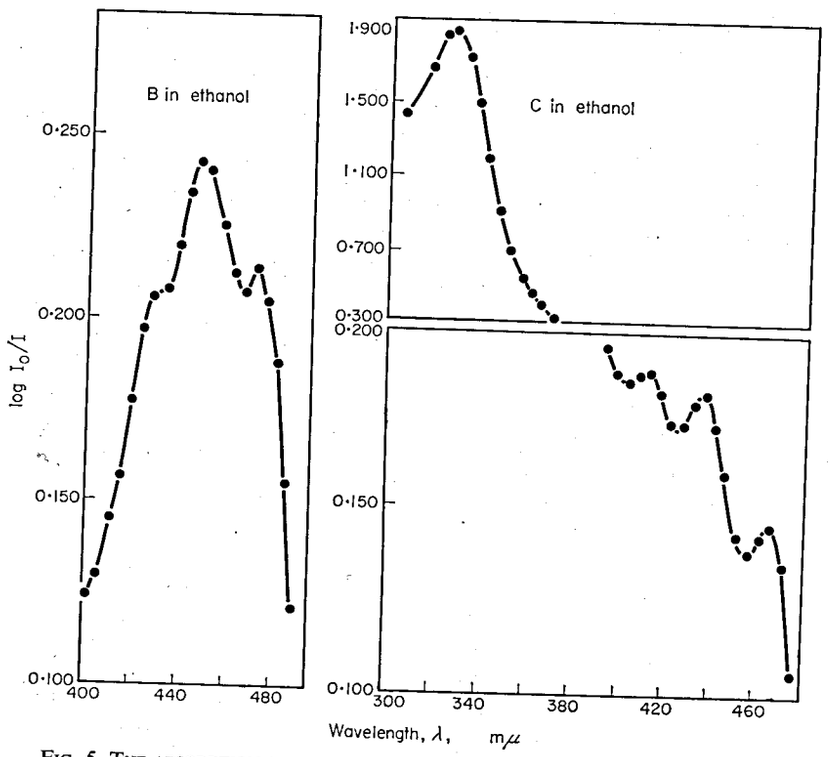

FIG. 5. THE ABSORPTION SPECTRA OF FRACTION B AND FRACTION C IN ETHANOL.

DISCUSSION

Yellow carrot varieties do not normally accumulate β-carotene, but they contain xanthophylls.[10] The situation observed in the red variety "Rouge longue géante de Flakée", when treated with GA_3 is similar, β-carotene is absent, but lutein is present. This shows that the inhibition of carotene accumulation in red carrots do not necessarily correspond to a simultaneous inhibition of xanthophyll accumulation. Some monocotyledons, etiolated in darkness, show the same phenomenon. Their tissues are devoid of any appreciable amount of carotene although zanthophylls are present.[11]

It is generally thought that xanthophylls are produced by oxidation of carotenoid hydrocarbons. Several facts support this view.[12] Possibly, the lutein found in GA_3 treated

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carrots arises by oxidation of α-carotene, only the synthesis of β-carotene being inhibited by GA_3 treatment. Our results also show that, in inhibiting the accumulation of β-carotene, GA_3 does not enhance the accumulation of lutein: the quantities of lutein found in the treated and control roots being of similar magnitude. It does not seem, therefore, that some precursor of β-carotene is used by the treated roots to form lutein, or any other pigment, except perhaps the C fraction.

The nature of the C fraction is unknown. Its absorption peak at 330 mμ, and its chromatographic behaviour indicate that it is not phytoene. It is possible that this fraction contains compounds which are normally utilized for the synthesis of β-carotene, or are alternatively oxidation products of the latter. In this case the observed decrease of β-carotene concentration would be due to its transformation into compounds of fraction C. These two alternatives require further investigation.

The fact that treated carrots are unable to accumulate β-carotene, is a clear example of an action of GA_3 on plant metabolism. Such an action has probably many implications; in particular, it is certainly related to the complex morphological and anatomical changes which have been described.

MATERIAL AND METHODS

Carrots of the variety "Rouge longue géante de Flakée" were sown in a greenhouse in loamy soil. Six weeks after sowing, they were divided into seven series (15 plants each). One series received distilled water (control); the other series received pure gibberellic acid (GA_3) at the following concentrations: 50, 100, 150, 200, 250 and 300 p.p.m., applied twice a week for 2 months. Each treatment consisted of applying 3 drops of the appropriate liquid to the bud of the plants.

At the end of the treatment the fresh weight of roots and foliage were determined, and the roots were analysed.

The amount of root carotenoids was measured at 460 mμ, in a chloroform extract of the whole tissue, and calculated as β-carotene (Gillam[13]). The pigments in petrol ether were chromatographed on a cellulose column (6 g, 1·5 × 10 cm) which had been washed twice with the solvent. Elution was carried out according to Costes;[14] first with petroleum ether; and then with increasing concentrations of acetone in petrol until pure acetone ran from the column. The eluate was collected in 1 ml fractions, and elution curves were drawn and the absorption measured for each fraction at 450 mμ.

The main fractions were identified after rechromatography on paper following the method of Chiba and Noguchi.[15] Absorption spectra in different solvents (methanol, ethyl alcohol, chloroform and carbon disulphide) were measured in a Beckman spectrophotometer.

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