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10. THE ROLE OF PLANT GROWTH REGULATORS IN THE DEVELOPMENT, GROWTH AND MATURATION OF THE FRUIT

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The study, begun in 1965, is being financed by a five-year grant from the U.S. Department of Agriculture. Its objective is to follow the changes in levels of growth regulators (auxins, gibberellins, cytokinins and inhibitors) during the development of avocado fruits, and to clarify the roles they play in the growth and maturation of the fruit.

a. Examination of Endogenous Growth Regulators in Fruit Tissues

Methods

Three bioassays were used for testing the level of regulator activity in methanolic extracts of the various fruit tissues: *(a)* the wheat coleoptile straight growth assay, to test auxins and coleoptile elongation-inhibiting substances; *(b)* the barley endosperm assay, to test gibberellin activity and the inhibitors of this activity; and (c) the growth of soybean callus in tissue culture, to test cytokinin activity and inhibitors of this activity.

Representative fruit samples of several varieties were harvested during fruit development. The following tissues were separated: mesocarp, seed coats, endosperm and embryo. The tissues were frozen and stored at -20°C, or freeze-dried and stored under vacuum until they were tested.

To extract the growth regulators the tissue was macerated in methanol and, after filtering, the extract was concentrated under low pressure to dryness. The concentrate was then dissolved in a small quantity of methanol and chromatographed on 3-cm-wide Whatman 3 MM strips. Each strip was spotted with a quantity of concentrate equivalent to 50-100 mg fresh tissue. The strips were chromatographed by ascending chromatography to a distance of 20 cm from the spotting line with isopropanol: ammonia: water (10:1:1) as solvent. The strip was then cut transversely into 10 or 20 equally wide sections. The activity in each section was assayed in the various tests.

To test auxin and elongation inhibitor activity, each paper section was placed in a vial (10 x 40 mm ID) with 0.75 ml potassium phosphate citrate buffer + 2% sucrose and three 10-mm wheat coleoptile sections. Incubation and elution of the active substances from the paper were conducted by continuous rotation of the vials at 24°C. After 22 hours the length of the coleoptile was measured.

In the gibberellin test each paper section was placed in a vial with 1.2 ml distilled water and two halves of embryo-less barley endosperm. The vials were rotated at 27°C for 32 hours. The amount of reducing sugar formed by amylase, which is activated by

gibberellin present in the paper, was determined at the end of the incubation period. The activity of gibberellin inhibitors was tested by the same method in the presence of gibberellic acid (GA_3).

Cytokinin activity was tested by putting the paper sections in the nutrient medium on which soybean callus was grown. Elution of the active fraction occurs during sterilization. Three soybean explants were planted in each vessel on 20 ml of medium. After four weeks' growth in the dark at 27°C, the weight of explants was determined. Under our working conditions, the soybean callus did not grow in the absence of cytokinins. With the addition of kinetin the growth was proportional to the concentration of kinetin added (from 0.005 to 5.0 ppm). Cytokinin inhibitor activity was found by determining the reduction of growth caused by the paper in a medium containing kinetin.

Results

The promotion and inhibition regions found in the three bioassays are summarized in Table B. 10. 1

TABLE B. 10.1

PROMOTION AND INHIBITION REGIONS FOUND ON PAPER CHROMATOGRAMS IN THREE BIOASSAYS

Bioassay	*Rf of chief promoting regions*	*Rf of chief inhibiting regions*
Wheat coleoptile elongation	0.2–0.4; 0.7–0.9	0.0–0.1; 0.6–0.7; 0.7–0.9
Barley endosperm	0.3–0.4; 0.6–0.7	0.6–0.7; 0.7–0.9
Soybean callus	0.0–0.1; 0.3–0.4; 0.6–0.7	0.7–0.9

The characteristics of substances active in different chromatogram sections, and their postulated identity, are as follows:

1. Auxin activity at Rf 0.2-0.4 was found in all tissues examined, but its level was very variable. The characteristics of the active substances strongly resemble those of indoleacetic acid (IAA).

2. Auxin activity at Rf 0.7-0.9 was generally found in the seed coats and endosperm and also in young embryos. The active substance is neutral and resembles indoleacetonitrile (IAN) in its characteristics.

3. Gibberellin-like activity at Rf 0.3-0.4 was found in seed tissues.

4. Gibberellin-like activity at Rf 0.6-0.7 was found only in the endosperm.

5, 6, 7. Cytokinin activity at Rfs 0.0-0.1, 0.3-0.4 and 0.6-0.7, respectively, were found in all seed tissues and in the mesocarp of young fruits.

8. Inhibitor A, at Rf 0.0-0.1, includes substances which do not move in the solvent used by us.

9. Inhibitor B, at Rf 0.6-0.7, inhibits elongation of coleoptiles and inhibits gibberellin action. In both cases the substance resembles abscisic acid in its behavior.

10. Inhibitor C, at Rf 0.7-0.9, appearing in mesocarp and embryos, strongly inhibits the elongation of wheat coleoptiles. Extracts from 50 mg fresh weight of mature mesocarp show very strong activity. This fraction exhibits moderate anti-gibberellic activity and also anti-cytokinin activity. The effect of growth inhibition of soybean callus caused by this substance can be reduced by raising the concentration of kinetin in the nutrient medium. Antibiotic activity against Gram-positive bacteria was also found in this fraction. The active substance is neutral and passes from aqueous solution to petrol ether.

Change in growth regulator levels in fruit tissues during development: Most of the tests were conducted with the variety Fuerte. Results obtained with other varieties were similar when fruits at similar stages of development were compared.

In general, we can summarize as follows: High levels of growth promotion activity were found in seed tissues, especially in the *seed coats* and *endosperm.* No reduction of activity level was found in the endosperm prior to its disappearance in early July. In contrast, activity of the seed coats diminished gradually with fruit development and maturation, disappearing completely by the time the seed coats (of Fuerte) shriveled and darkened in September. The rate of fruit growth slowed down at the same period (Fig. B. 10. 1), and rapid oil accumulation began.

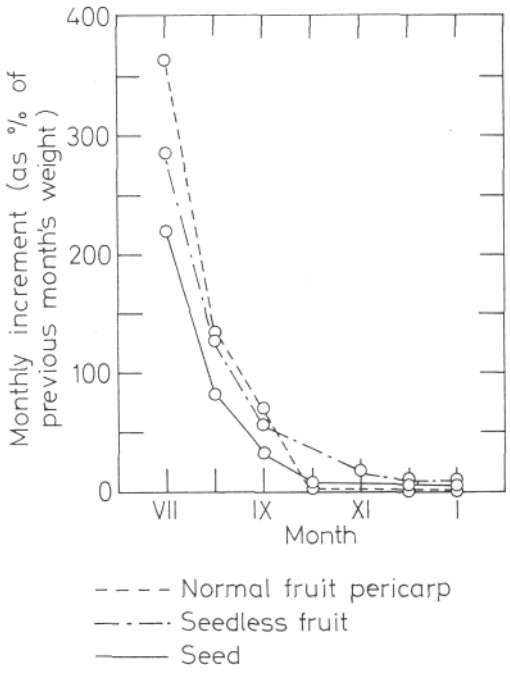

Fig. B.10.1. Relative growth rate of Fuerte fruits and seeds.

Embryo (cotyledons): The activity of auxins and gibberellins was moderate to weak, mainly at the stage when it was growing rapidly. In contrast, the activity of cytokinins was very marked at all stages of seed development. Some activity was found even when the fruit had matured and the seed had no connection with the flesh. From August on, very strong inhibiting activity was found at Rf 0.8-0.9.

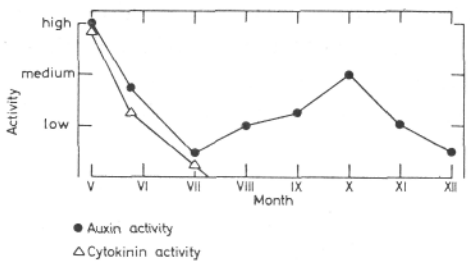

Fig. B.10.2. Levels of auxin and cytokinin activity in Fuerte mesocarp extract during fruit development.

Mesocarp: No consistent relationship between the activity of growth promoters (Fig. B. 10.2) and the rates of growth and development (Fig. B. 10.1) could be found. Auxin and cytokinin activity levels were found to be high in very young fruits. The activity declined rapidly and auxin levels were very low by July. A wave of fruit drop occurred in July, and a connection between the two phenomena may exist. Auxin activity increased again, reaching a peak when the fruit matured and its growth rate was very slow. As winter approached, the level declined.

Cytokinin activity in the crude mesocarp extract disappeared in July, a time when the fruit was growing rapidly. When the extract was separated on an acid column or was acid-hydrolyzed, cytokinin activity appeared.

The level of gibberellin activity was very low in the mesocarp and no fluctuations in the level could be discerned during fruit development. In contrast, a constant increase in the activity of inhibitor C was found with the development of the fruit and decrease of its growth rate (Fig. B. 10. 3).

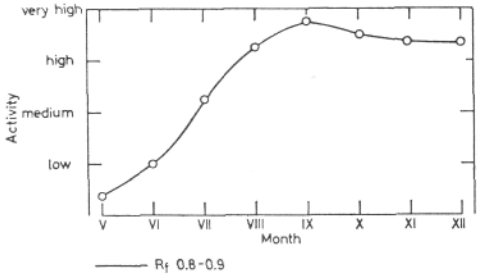

Fig. B.10.3. Levels of activity of inhibitor C in Fuerte mesocarp extract during fruit development.

b. The Relationship between the Growth, Development and Maturation of the Fruit, and Growth Regulator Activity

High levels of auxins, gibberellins and cytokinins were found in the seed coats, endosperm and young embryo. Extremely high levels were found in the seed coats and endosperm. The latter constitutes a large proportion of the young fruit, diminishing during development in relation to the rest of the fruit, and finally disappearing about

three months after fruit set. At this stage the highest level of activity is found in the seed coats. As the growth rate of the fruit diminishes (Fig. B. 10.4), the level of regulator activity in the seed coats declines. Fuerte fruit reaches horticultural maturity in September, its growth rate then reaching a minimum. Oil accumulation is very rapid at this stage. Concurrently, the seed coats degenerate and growth regulator activity disappears. At this stage, the embryo also ceases growth. We can summarize that there is a definite positive relationship between the high level of growth regulator activity in the endosperm and seed coats, and rate of fruit growth. We cannot establish whether these tissues provide a source of growth regulators for the growing mesocarp, or whether their main function is to enhance the flow of photosynthates from the leaves into the fruit.

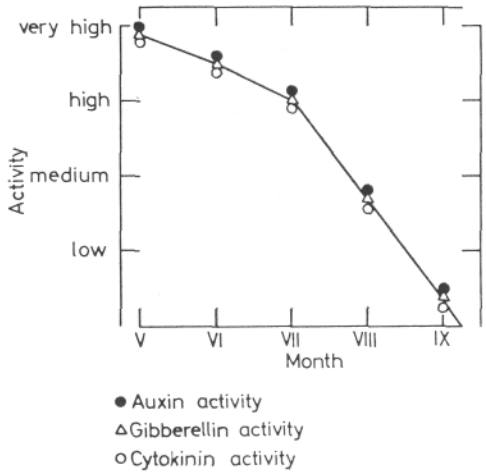

Fig. B.10.4. Levels of auxin, gibberellin and cytokinin activity in Fuerte seed coat extract during fruit development.

It is interesting to note that the presence of extractable auxins, cytokinins and gibberellins in the mesocarp, is not a prerequisite for its rapid growth rate and meristematic activity. This is especially conspicuous in July, when the mesocarp is growing rapidly while the level of the regulators is very low.

The negative correlation between the level of inhibitor C in the mesocarp and its growth rate, should be noted. We cannot yet determine whether this is a causal correlation or whether this substance just accumulates during fruit growth and development.

c. The Influence of Exogenous Growth Regulators on Fruit Growth and Development

Our main efforts this year were centered around trials to get various synthetic and natural growth regulators into the fruit. Only if changes in fruit development can be brought about by altering the level of growth regulators in the fruit, can the role of growth regulators in fruit development be proven. Otherwise, it is not possible to determine whether the correlations found between endogenous regulators and fruit growth are of the cause and effect type, or not.