

Cytokinin Activity in Avocado Seeds during Fruit Development^{1,2}

Received for publication December 8, 1969

A. BLUMENFELD³ AND S. GAZIT

Department of Horticulture, The Volcani Institute of Agricultural Research, Bet Dagan, Israel; and the Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot, Israel

ABSTRACT

The soybean callus bioassay was used to determine levels of cytokinin activity in avocado (*Persea americana*) seeds.

In the embryo, levels are high during the early stages of development, but diminish as the fruit grows. The level of cytokinin activity in the endosperm is very high throughout the period that this tissue exists. The seed coats have very high activity levels while the fruit is young, reaching values comparable with those found in the endosperm. The activity level falls as the rate of fruit growth slows down and disappears completely by the time the seed coats shrivel at approximately the same time the fruit reaches "horticultural maturity".

coats (integuments), endosperm (when found), and embryo were separated. The samples were cut up into pieces of approximately 0.2 g; then they were frozen in liquid air and freeze-dried in bottles containing 5 or 10 g of the material. When drying was complete, the bottles were sealed under vacuum and were stored. There was a time interval of approximately 2 hr from the time of picking until freezing.

The dried samples were extracted with 80% methanol; after filtration, the methanol was evaporated. The aqueous residue was first shaken with petroleum ether, then acidified to pH 2.5, and shaken with ethyl acetate to remove inhibitors (2, 4). The acidic aqueous fraction was chromatographed by ascending paper chromatography by using isopropanol-ammonia-water (10:1:1) as the solvent. The chromatogram strips were cut transversely into 10 equal sections, and the cytokinin activity of each was assayed by Miller's soybean bioassay technique (6).

RESULTS

DEVELOPMENT OF THE DIFFERENT SEED TISSUES

The seeds greatly influence the development of many fruits. In some, the rate of fruit growth decreases during periods of rapid growth of the embryo (10). Fruit shape is sometimes influenced by the presence or absence of seeds, or by their number (9). In most plants, the fruit does not grow without the development of normal seeds, but a number of important species constitute exceptions to this general rule. How seeds influence fruit development is not clearly understood, but it is usually assumed that one of the main factors is via growth substances which generally occur in the seed at high levels of activity. The presence of auxins, gibberellins, and cytokinins (8) has been demonstrated in seeds of many fruit species; however, no correlation between the level of activity of these substances in seeds and the development of fruits could be established in many cases. In the present study, we have explored the relationship between cytokinin activity in avocado seeds and fruit growth. The results of our studies on other growth substances will be published separately.

MATERIALS AND METHODS

Avocado fruits (*Persea americana* Mill) of the varieties Northrop, Glickson, Fuerte, Hass, Nabal, and Booth-8 were picked 4 days after irrigation from trees carrying a good crop. Horticultural maturity is reached in Northrop and Glickson in August; in Fuerte, in October; in Hass, in November; and in Nabal and Booth-8, in January.

The fruit was opened in the laboratory, and the pericarp, seed

Seed Coats. The avocado seed is bitegumental, but it is difficult to distinguish between the two integuments (1). These integuments constitute 4.5% of the weight of a young Fuerte fruit in June, and increase in weight until August. At this time, the seed coats begin to shrink and gradually dry up (Table I). The fruit reaches horticultural maturity when the seed coats shrivel.

Endosperm. The endosperm completely envelops the embryo during the first 2 or 3 months after fruit set. It subsequently disappears gradually.

Embryo. The avocado has a single, relatively large embryo (Table I) consisting of two large, starchy cotyledons; the radicle and plumule remain very small, and even at maturity they weigh only approximately 0.1 g. The Fuerte embryo continues growing until September, when its seed coats shrivel.

ENDOGENOUS CYTOKININS

Cytokinin activity was similar in fresh and preserved fruit extract. When substances having cytokinin activity were loaded on Dowex 50 (H⁺) ion exchange columns, the activity passed into the ammonia eluant (2).

Zones of Activity. Three zones of cytokinin activity were found on paper strips examined after chromatography (isopropanol-ammonia-water (10:1:1), at R_F values 0.0 to 0.2, 0.3 to 0.4, and 0.7 to 0.8).

Cytokinins from the Endosperm. Levels of cytokinin activity in the endosperm were very high. Concentrations equivalent to 2 g fresh weight per 100 ml of nutrient medium induced considerably more callus growth than 0.5 mg/liter of kinetin. When the extract concentration was reduced 10-fold, a marked response was still produced (Fig. 1).

Cytokinin activity of the endosperm was maintained at a very high level as long as the tissue was present in the seed.

¹ Contribution from the Volcani Institute of Agricultural Research, Bet Dagan, Israel. 1969 Series, No. 1627-E.

² This research was financed in part by a grant from the United States Department of Agriculture under Public Law 480; United States Department of Agriculture Grant FG-Is-206.

³ Present address: Michigan State University, East Lansing, Michigan 48823.

Cytokinins from the Seed Coats. Three zones of activity were investigated; the most active fraction was found at R_F 0.0 to 0.2. Activity levels were very high during the early stages of fruit development and reached values similar to those found in the endosperm; activity diminished during fruit development and disappeared completely by the time the seed coats shriveled and the fruit matured (Fig. 2). This pattern of change in the level of activity was found in all of the varieties tested.

When fruit was harvested at the beginning of August, the level of cytokinin activity was relatively high in the late maturing Nabal and Booth-8 varieties; it was moderate in the midseason-maturing varieties and was low in the early variety, Northrop, which was then close to maturity.

Cytokinins from the Embryo. The level of activity at R_F 0.1 to 0.2 was lower than at R_F 0.4 to 0.5, in contrast to the findings in endosperm and seed coats. Variations in the level of cytokinin activity in the embryo over the seasons are shown in Figure 3. It was high at early stages of development, showing a gradual decrease as the fruit matured. However, a relatively high level remained in the mature fruit when the embryo was disconnected from the pericarp. Weak cytokinin activity was found in Fuerte embryos even in February, at least 4 months after fruits were horticulturally mature and the seeds were completely independent. Cytokinin activity in the embryo was mainly due to activity in the cotyledons. Activity levels were found to be identical

Table I. Weights of Fuerte Fruits and Their Components, Picked during the Period June to November, 1968

Picking Date	Average Fruit Weight	Average Pulp Weight	Average Weight of Seed		
			Endo-sperm	Seed coats	Embryo
	g	g	g	g	g
June 25	32.8	28.4	0.4	1.5	2.5
July 23	102.4	87.0	0.4	3.5	11.5
August 21	158.9	137.0		4.3	17.6
September 25	213.4	176.3		3.1	33.9
November 5	230.6	193.1			37.0

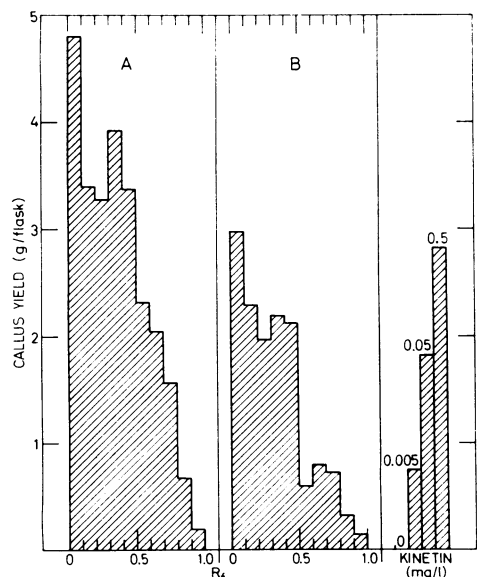


FIG. 1. Cytokinin-like activity of avocado endosperm. A: Extract of 2 g of endosperm per 100 ml of nutrient medium; B: extract of 0.2 g of endosperm per 100 ml of nutrient medium; last section: equivalent activity of kinetin under the same conditions.

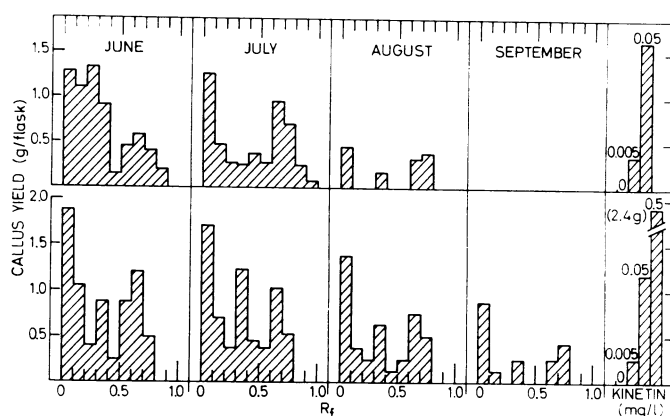


FIG. 2. Response of soybean callus to extracts of seed coats, equivalent to 2 g fresh tissue per 100 ml of nutrient medium. The month during which the fruit was harvested is indicated. Upper row: Early maturing variety (Glickson); lower row: midseason-maturing variety (Fuerte); last section: equivalent activity of kinetin under the same conditions.

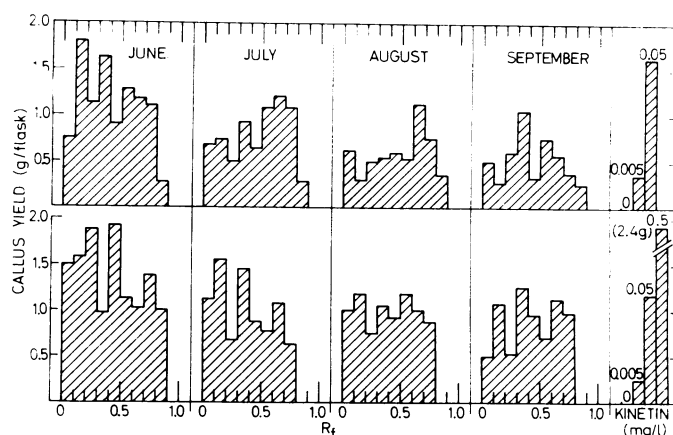


FIG. 3. Response of soybean callus to embryo extract, equivalent to 5 g fresh tissue per 100 ml of nutrient medium. The month during which the fruit was harvested is indicated. Upper row: Early maturing variety (Glickson); lower row: midseason-maturing variety (Fuerte); last section: equivalent activity of kinetin under the same conditions.

when extracts of cotyledons alone or of whole embryos were tested. When extracts of the radicles and plumules, equivalent to 0.5 g fresh weight of the material, were added to 100 ml of nutrient medium, no cytokinin activity was detected.

DISCUSSION

The seed plays an important role in the development of the avocado fruit, as is evidenced from the fact that seed-bearing fruits are much larger (10-fold) than seedless fruits. The fact that shriveling and darkening of the seed coats at an early stage of fruit development are connected with young fruit drop emphasized the importance of these tissues.

A positive correlation was found to exist between the rate of fruit growth and the level of endogenous cytokinins in the seed tissues. In the early stages, 2 or 3 weeks after fruit set, the endosperm, together with the seed coats and the embryo, forms the main reservoir of growth regulators in the fruit. The endosperm disappears at a time when the fruit is still growing rapidly. At this stage the activity of the seed coats remains very high, whereas the activity in the embryo is moderate. As the fruit

matures, the seed coats shrivel and their activity diminishes and finally disappears.

The level of cytokinins in the seed coats was found to depend on the physiological age of the fruit, not on the season. Tests of early and late varieties showed a similar trend in the level of activity although the former reach maturity in summer and the latter, in autumn or winter.

Because relatively high rates of activity were still found in the embryo at the stage when the fruit had reached maturity and the seed coats had shriveled, the seed may be considered to be not only a reservoir of growth regulators for the developing fruit and also a sink for drawing nutrients, etc., to the fruit, but also a reservoir of cytokinins for the germinating seedlings.

High levels of cytokinins are usually found in seed when compared to other fruit tissues (5). This also seems to be true for avocado (2). The relative abundance of cytokinins in apple seed has been considered by Letham (5) as an indication that the apple seed is the principal center of cytokinin biosynthesis in this fruit. Although we tend to agree with this conclusion, there seems to be a lack of direct evidence of cytokinin production in the seed. The fruit is continuously supplied with cytokinins from the sap (3, 5), and the relative importance of the role of

these two cytokinin supply sources to the fruit has not yet been defined.

LITERATURE CITED

1. CUMMINGS, K. AND C. A. SCHROEDER. 1942. Anatomy of the avocado fruit. Yearbook of the California Avocado Society 1942. pp. 56-64.
2. GAZIT, S. AND A. BLUMENFELD. 1970. Cytokinins and inhibitors activity in the avocado fruit mesocarp. *Plant Physiol.* 46: 334-336.
3. KENDE, H. 1965. Kinetin-like factors in the root exudate of sunflowers. *Proc. Nat. Acad. Sci. U. S. A.* 53: 1302-1307.
4. LETHAM, D. S. 1963. Regulators of cell division in plant tissues. I. Inhibitors and stimulants of cell division in developing fruits. Their properties and activity in relation to the cell division period. *N. Z. J. Bot.* 1: 336-350.
5. LETHAM, D. S. 1969. Regulators of cell division in plant tissues. VIII. The cytokinins of the apple fruit. *Physiol. Plant.* 22: 925-36.
6. MILLER, C. O. 1963. Kinetin and kinetin-like compounds. *In: Modern Methods of Plant Analysis*, Springer Verlag, Berlin. Vol. 6. 194-202.
7. MILLER, C. O. 1967. Cytokinin in *Zea mays*. *Ann. N. Y. Acad. Sci.* 144: 251-257.
8. NITSCH, J. P. 1965. Physiology of flower and fruit development. *In: Encyclopedia of Plant Physiology*, Vol. 15, Part 1. Springer Verlag, Berlin. pp. 1537-1647.
9. ROBERTS, R. H. 1946. Notes on apple set and growth. *Proc. Amer. Soc. Hort. Sci.* 48: 59-62.
10. TUKAY, H. B. 1934. Growth of the peach embryo in relation to growth of fruit and season of ripening. *Proc. Amer. Soc. Hort. Sci.* 30: 209-218.