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CHANGES IN ENDOGENOUS HORMONE ACTIVITY DURING PARTHENOCARPIC AND NON-PARTHENOCARPIC SYCONIUM DEVELPMENT OF *FICUS CARICA* L., Cv. KING.

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Abstract

To obtain information concerning relative activities of native hormones during development of fig syconia in the first (parthenocarpic) and second (nonparthenocarpic) crops of the King cultivar, the following procedure was tested. It was based on the assumption that the growth response of fruit wall explants from a sequence of syconium samples to the same concentration of an exogenous growth regulator incorporated in the culture media would be inversely related to the 'activity' (or content available for growth processes) of the corresponding endogenous type of hormone in fruit wall tissue at time of sampling. Thus, if much growth were induced by a regulator the probable level of the corresponding native type of hormone available was considered to be low and *vice versa*. The regulators used were 2,4-D, kinetin, and gibberellic acid, each at two concentrations pre-determined as suitable. The means of one month's explant growth from the two concentrations were plotted inversely against time to show the changes in relative endogenous hormone 'activity' during fruit development. The results were correlated with data for extracted free auxins and acidic gibberellins from the same fruit samples, and also with curves of weekly growth increment. Evidence of 'sequential growth' was found. In the first crop auxin activity was highest in the early rapid growth phase. Cytokinins were highest in the early part of the slow growth phase, when supernumerary ovules in the first crop and endosperm and embryos in the second were developing. Gibberellins were highest in the later part of the slow growth phase in both crops. In the second crop, auxin activity as well as extracted auxin levels were much lower throughout development than in the first crop, and gibberllins were generally higher.

Introduction

Crane *et al* (1959) extracted free auxins from second-crop figs of Calimyrna (non-parthenocarpic) and Mission (parthenocarpic) cultivars sampled periodically during development. No correlation was found between concentrations of free auxins and fruit growth rates. In view of their findings, no correlation was anticipated between total free auxin concentrations and rate of fruit growth in the King fig, which proved to be the case (Lodhi *et al*, 1969) In order to obtain some information concerning growth hormones active in fruit growth processes and their changes in activity during fruit development, an indirect approach was tested.

The assumption was made that the response of fruit wall explants from a succession of syconium samples to the same concentrations of an exogenous growth regulator incorporated in the culture media would be inversely related to the activity (or content available for growth) of the corresponding native type of hormone in fruit wall tissue at the time of fruit sampling. Thus, if much growth were induced by a regulator, the probable level of the corresponding native type of hormone available was considered to be low, and *vice versa*.

In view of the general assumption that interactions occur among endogenous hormones of the three major categories (auxins, gibberellins, and cytokinis), it is recognized that the growth stimulating effects of an exogenous regulator would probably involve interactions between it and native hormones of one or both of the other two categories. The extent of growth would be expected to depend also on activities of inhibitors of the type of endogenous hormone represented by the exogenous regulator as well as inhibitors of the endogenous hormones with which the exogenous ones would interact. Thus, the exogenous hormone would be active in growth to the extent permitted by the milieu of native hormones and inhibitors, and also of substrates for a variety of substances in the synthesis of which the hormones and inhibitors would participate. Nevertheless, if much explant growth were stimulated by an exogenous hormone, that could be taken to mean that the content of availability of the corresponding native hormone(s) was at least one of the factors restricting maximum growth. If, on the other hand, no or practically no explant growth were induced, that should incicate that within the limits of the hormone-inhibitor-substrate environment, the supply of the endogenous hrmone(s) in question was not limiting.

Admittedly, the growth of explants does not reproduce growth of the tissue in the fruit. Nevertheless, the same processes of tissue growth are duplicated, that is, the processes involved in cell division, and in cell enlargement with or without accompanying endopolyploidization (Lodhi & Bradley, unpublished). The types of growth may be influenced in different directions depending on the cateogry to which an applied regulator belongs, as shown by Gautheret (1959) and many others.

Materials and Methods

Syconia of both first and second crops were collected for two years at 2-week intervals on the same calendar dates. The collections coincided with samples taken on alternate dates for extraction of auxins and gibberellins (Lodhi *et al* 1969), so that the results from extractions could be compared with those from explant experiments. Collections of first year were used for tests of the suitability of the culture medium with and without growth substances. Explants from ripening figs in growth period III could not be cultured successfully. Fruit samples were held in a refrigerator and were cultured within a few days after collection.

The basic culture medium contained one-half the concentration of macro- and micro-elements in Murashige and Skoog's modification (1962) of White's medium (1943). Choline chloride (0.5 mg/l) was added to their list of vitamins, and 2% instead of 3% sucrose was used. The source of iron was Fe-EDDHA (sodium ferric ethylene diamine di-(0-hydroxyphenyl acetate). Bacto-agar (0.8%) was added to solidify the medium. pH of the medium was adjusted to 6.2 before injection of 10 ml aliquots of medium into 15x150 mm test tubes, which were then

autoclaved (20 min. 15 Psi. 120°C). 'KN'—Kinetin (6-furfuryl amino purine) and 2, 4-D'—(2, 4, dichlorophenoxyacetic acid) were added to the medium before autoclaving, 'GA'—gibberellin (K salt of gibberellic acid) was simultaneously sterilized and injected into test tubes of warm, unsolidified medium with a Cornwall automatic pipetting apparatus including a B-D Swinney adapter. The following concentrations were used: 0.1 ug/ml KN, 0.5 ug/ml 2, 4-D, and 10 ug/ml GA, and also doubled concentrations of each. Previous tests had shown these concentrations to be most suitable. In addition, all possible combinations of the growth regulators at the lower, and at the higher concentrations, respectively, were tested. The basic medium without growth substances was used for control explants.

In preparation for culture, the surface of the fruits was sterilized with 0.3% Na-hypochlorite solution for 15 min. followed by washing in sterile water. The fruits were cut in half, the outer surface pered, and buds or flowers and the intervening epidermis were removed aseptically. Small square segments were cut from the region of maximum horizontal fruit diameter, and each was weighed. Fifteen to 20 replicate cultures were grown for each tests.

After four weeks in culture, the percent mean fresh weight increment for each control and growth regulator test was calculated, and the net percent increment determined (% increment from a regulator test minus the value from the corresponding control). The value plotted for each regulator per fruit sample was the mean of the net percent increments induced by the two concentrations used.

As mentioned before, the procedures adopted were based on the assumption that the response of syconium explants of any given fruit sample to medium containing a particular exogenous regulator would be inversely related to the activity (or content available for growth) of the corresponding native hormone(s) in syconium available for growth) of the corresponding native hormone(s) in syconium tissue at the time of fruit collection. The curves of endogenous hormone "activity" were therefore plotted as the reciprocals of curves of growth increment of explants treated with corresponding exogonous hormone (Fig. 1). The reader should be cautioned that the figures along the ordinate pertain *only* to net percentages of explant growth. The activity curves are not intended to indicate anything more than *relative* levels of activity of the three types of endogenous hormone on any particular date of fruit sampling, and changes in *relative* activity of one hormone on different dates.

Results and Discussion

Although fruits of the first crop grew more rapidly than those of the second crop throughout development, and attained a size approximately one and one-half times larger than the latter, control explants from second-crop fruits grew much more than those of the first crop (Table 1). The differences in growth might be interpreted in various ways, of which the following possibilities are suggested: (1) Fruits of the second crop may have been relatively deficient in non-hormonal substances needed for maximum growth but supplied by the control medium. Evidence in support of this possibility concerns competition of the two crops with shoot growth and with one another. Although the first crop competed with shoot growth throughout fruit development, competition began at the start of the growing season, when an abundance of materials stored in roots was available. The second crop competed with shoot growth for the first two or three weeks of fruit develop-

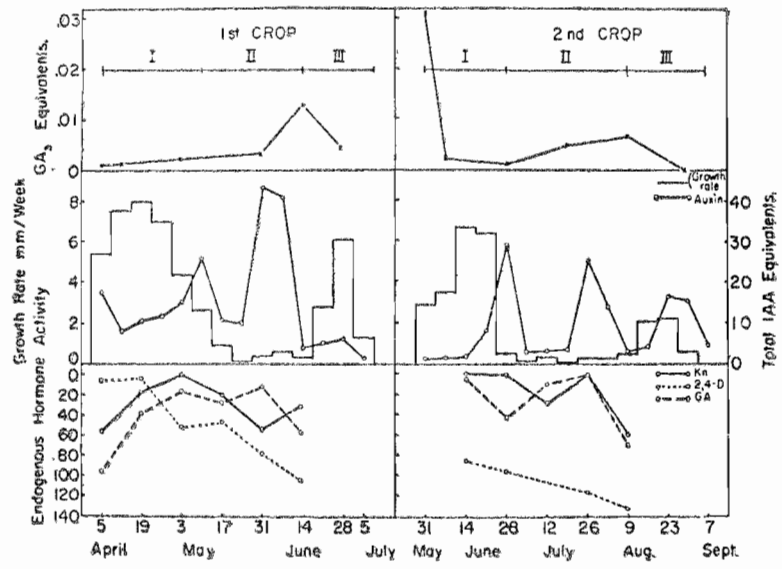

Fig. 1. Top: Duration of fruit growth periods I, II, and III in both crops; and changes during the season in concentrations of acidic gibberellins extracted from fig syconia of first (parthenocarpic) and second (non-parthencarpic) crops. Middle: weekly fruit growth increments; and changes in concentrations of total 'free' auxins extracted from first—and second—crop figs. Bottom: Changes in relative 'activities' of endogenous auxins, cytokinins, and gibberellins during development of the two fig crops. The 'activities' of a hormone are inversely related to response of explants of fruit wall tissue to culture medium containing the corresponding exogenous hormone. Therefore, the ordinates are the net % growth increments in inverse order. In noting relative activities of a hormone, the ordinates should be ignored.

ment, but perhaps more important was competition with first-crop figs during the first five or six weeks. During almost all of the latter period, first-crop figs were in the "final swell", when sugars and other substances were flowing into them, which no doubt meant deprivation for the second crop. (2) Second-crop fruits may have contained inhibitors not present in the first crop, which were sufficiently diluted by the liquid in the medium or otherwise inactivated by substances—therein to enable much growth to occur. (3) Fruits of the second crop may have contained more endogenous hormones than first-crop fruits. Arguing against this was the response of explanats from fruits of the two crops to the combination of all three exogenous regulators in the culture medium; those from the second crop consistently showed a much greater response than explants from first-crop fruits (Table 1).

The additions of combinations of two or three growth regulators to the medium were useless in throwing any light on the activity of individual endogenous hormones. Because of the possibility of interactions with endogenous hormones as well as among themselves, any interpretation seemed nebulous.

TABLE. I. Growth of explants on basic medium (I), and on basic medium to which the combination of kinetin, 2,4-D, and gibberellic acid was added (II).

Collection dates	I[1]	II[2]
1st crop		
April 5	28.8	73.0
April 19	20.4	95.5
May 3	28.8	21.6
May 17	20.0	140.0
May 31	27.0	136.9
June 14	28.5	122.4
2nd crop		
June 14	124.7	241.0
June 28	44.0	155.8
July 12	63.1	269.8
July 26	101.1	166.7
August 9	76.2	156.5

[1] Values were % increases in fresh wt. [2]Values are net % increase in fresh wt. (% increases in fresh wt. on medium containing the combination of regulators minus corresponding control values).

To facilitate correlation of changes in native hormone activity with changes in extracted free auxins and acidic gibberellins, and with changes in fruit growth rates, the evidence concerning all these aspects are brought together in Figure 1. Also included are the curves of individual extracted auxins (Fig. 2) since it is necessary to refer to them in connection with endogenous hormone activity.

The patterns of activity of the three endogenous hormones differed. In the first crop, the highest activity among the three shifted from one to another during fruit growth, thus lending support to van Overbeek's hypothesis of "equentials growth" (van Overbeek, 1962). That hypothesis was based on the work of Wright (1961) who found that the sensitivity of wheat coleoptile sections to the exogenous growth regulators indoleacetic acid, kinetin, and gibberellin changed with age of the seedling. During the initial period or rapid syconium growth, auxin activity was higher than activity of the other two hormones and may be considered to have been most influential in that phase of growth. Cytokinin activity, which has been increasing from a moderate level while auxin activity was high, was ascendant as activity of auxins decreased and as syconium growth slowed. The approximately four weeks during which it was highest coincided with the most rapid phase of supernumerary ovule development and the cell division associated with it. Gibberellin activity, very low in the first sample, increased rapidly while the other two hormones were

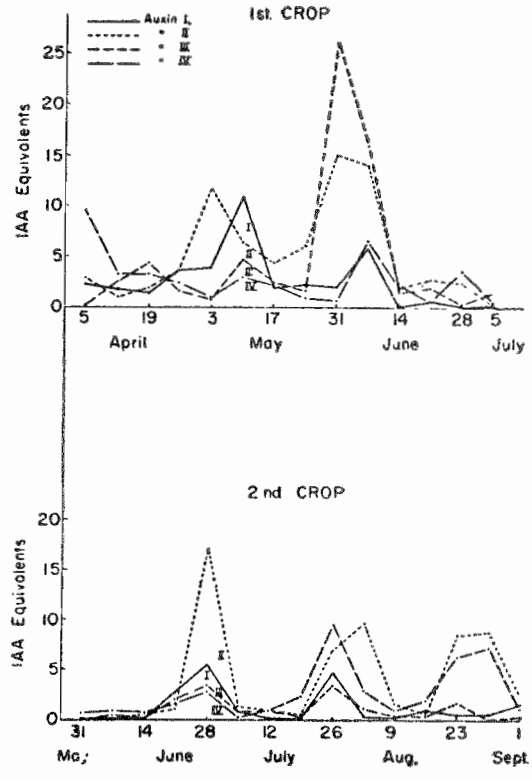

Fig. 2. Changes during fruit growth in concentrations of each of the four auxins extracted from first—and second—crop figs, as determined by growth-promoting activity of crude methanol extracts on wheat coleoptile sections. IAA equivalents are per gm dry wt of syconia tissue.

in their most active phases, and with the decrease in cytokinin activity, gibberellins became preeminent. While their activity was highest, fruit growth was in the slowest phase.

In the second crop, sequential growth was less certain in so far as ascendancy of auxins was concerned because of lack of a sample taken in a fruit growth phase comparable to that of the first sample in the first crop. In view of the extremely low auxin activity and the very high levels of gibberellin and cytokinis in the first sample, it is doubtful that auxin activity in the two weeks before the first sample was sufficiently high to have been dominant. Ascendancy of cytokinin before gibberellin activity conformed to the sequence in the first crop.

Auxin activitity in the first crop followed a course that was the reverse of the pattern of extracted free auxin II up to May 31, after which both auxin acitivity and auxin II concentration decreaseed (cf. Fig. 1&2). Thus, auxin II rose to its first peak while auxin activity was falling from the initial and highest level: and the peak

level of auxin II and the end of the decline of auxin activity occurred on the same date (May 3). Auxin activity then rose slightly while auxin II dropped from the peak, then resumed its descent while auxin II increased to the second peak, after which both decreased. In the second crop, the only evidence obtainable was th. decrease in auxin activity while all four extracted auxins increased to the first peake Absence of the third sample obscured any changes that might have occurred the second and fourth sampling dates. The relationship between the changes in direction of extracted auxin II and endogenonous auxin activity suggest that auxin II was primarily responsible for fruit growth in the first six or seven weeks at least. It may also be evidence that the extracted auxins represented formerly bound and continuous liberation of bound auxins from the second sampling date through period II may have accounted for the higher second than first peaks of three of the four individual auxins in the first crop (Fig. 2). Significantly, the decrease in fruit growth rate and in native auxin activity began at the same time in the first crop. At that time also, extracted auxins I and II began increasing toward peak levels. In the second crop, auxin activity, extracted auxin levels, and the rate of fruit growth were generally considerably lower than at comparable phases of fruit development in the first crop. As in the first crop. auxin activity decreased as extracted free auxin (all four in this case) increased to the first peaks, and as the fruit growth rate diminished.

Cytokinin activity in the two crops was dominant during the period of most rapid supernumerary ovule development in the first crop, as mentioned before, and in the second crop during early endosperm and embyro development. In the second crop, the trough in the cytokinin curve corresponded with the third period of endocarp lignification, during which embyro and endosperm develpment was practically at a standstill. Therefore, development of those structures was completed as cytokinin activity reached a peak and then decreased. In the first crop, supernumerary ovule development continued at least through period II, but at a diminishing rate in the last few weeks during which a trough appears in the curve and in which period endocarp tissue was being lignified. The rise at the end of the curve could have been related to a resumed higher rate of supernumerary ovule development and endopolyploidization of cells of both 'seed' and fruit wall tissues.

Gibberellin activity became dominant early in period II, and continued at high levels in both crops for about three weeks. The curves in the two crops tended to parallel the respective curves of extracted acidic gibberellins. in direction but not degree, until after the middle of period II. In the first samples of the first crop, both gibberellin activity and extracted gibberellins were at very low levels and thereafter increased in general during the following eight weeks. A slight trough appeared in the gibberellin activity curve during part of that period, however, and the timing of the peaks of gibberellin activity and extracted gibberellins was different, that of gibberellin activity preceding the peak of extracted gibberellin concentration by two weeks. In the second crop by contrast, gibberellin activity was high in the first sample, suggesting that it may have been falling from a much higher level, in conformity with a drop from the initial high level of extracted gibberellins. In the intervals between the first and second samples gibberellin activity decreased, just as extracted gibberellins decreased somewhat during that period. In the following four weeks both increased. But as in the first crop, while gibberellin activity decreased thereafter, extracted gibberellin concentration continued rising to a peak two weeks later and then fell to zero in the next two weeks. Thus, although the latter part of the curves of gibberellin activity in the two crops were similar, and

likewise those of extracted gibberellins, they were unlike in the early phase of development. Gibberellin activity was dominant in the same part of period II in both crops. It may be significant that it was higher than that of the other two honomones during lignification of endocarp, though we are not aware of reports of participation of gibberellins in lignification.

Just as extracted auxin and gibberellin levels moved in opposite directions in the latter part of period II and period III in both crops, so that levels of auxin and gibberellin activity proceeded in opposite directions in the first crop from the second sampling date until May 31, after which the activities of both decreased. In the second crop, however, the decreasing auxin activity in the first two weeks of fruit sampling was paralleled by decreasing gibberellin in that period, after which the latter increased while auxin activity declined, as in the first crop.

The coincidence of changes in direction of three or four of the extracted hormone and hormone activity curves appears to be evidence of either interactions among the three types of hormone, or of the influence of unknown factors, perhaps inhibitors. Four of the five curves changed direction on May, including that of auxin II of the four extracted auxin (Fig. 2), four on May 17, and four on May 31 in the first crop. In the second crop, four such changes took place on June 28, and three on July 26. As concerns the changes on May 31 in the first crop and July 26 in the second, the paper by Hirai (1966) shows other types of events in the second non-parthenocarpic) crop of the Masui Dauphine cultivar of the fig at the same phase of development as on July 26 in the King fig, and, by implication, as on May 31 in the first crop. They included (1) a slight peak of respiration and also one of malic acid in fruit wall tissue, and much higher peaks of both in the fruitlets, (2) a peak of reducing sugars in the fruits, and peaks of starch in both fruit wall and fruitlets, (4) beginning of rapid increase of pelargonidin, and (4) a peak of chlorophylls a and b. With all those changes at that time, and perhaps others in other systems, those in the growth hormone patterns appeared to be just one phase of a general change. The predominance of downward trends in curves of both crops at that time, with only extracted gibberellins increasing in both, suggest that the stage was being set for onset of period III and the different type of growth therein.

The differences in growth increments in the two crops of the King fig may be explained partly by the major differences in their hormone constitution. The major differences, and no doubt those which affected growth the most, were (1) the much lower extracted auxins and also auxin activity in the second than the first crop, and (2) exceptionally high extracted acidic gibberellin concentration and likewise high gibberellin activity in the second crop early in development. The fact that the growth rate in period I in the second crop was low until after the extracted gibberellin concentration had dropped to a low from the extremely high level suggests that the high concentration had had a somewhat inhibiting effect on growth. By contrast, in the first crop, which exhibited much greater growth in period I, both extracted gibberellins and gibberellin activity were low in the early growth phases while both extracted auxins and auxin activity were high. It is possible, however, that another factor in the inferior growth of fruits of the second crop was deficiency in nutritive substances, which may have accounted for the considereably greater growth on the control medium of explants from the second than the first crop.

In spite of the indirect nature of the growth hormone activity approach to determining something of the relative actions of endogenous auxins, cytokinis, and gibberellins within the fruits at different phase of fruit development, it seems to have merit when used in conjunction with data concerning changes in extracted hormones and in growth rates. The differences between the crops as concerns the auxin and gibberellin activity curves are consistent with the evidence from extracted auxins and gibberellins, and the changes in ascendancy of activity from one hormone to another are consistent with the changes in sites of the most active growth, that, is, fruit wall vs. seed structures.

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