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HISTOLOGICAL EXPRESSION OF IN VITRO GROWTH OF EXPLANTS FROM PARTHENOCARPIC NONPARTHENOCARPIC SYCONIA OF FICUS CARICA L., CV. KING

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Abstract. *In vivo* growth of both parthenocarpic (first crop) and pollinated (second crop) syconia of *Ficus carica* cv. King proceeded in three stages, two stages of rapid growth (period I and III) separated by a period of slow growth (period II). To obtain information concerning relative sensitivity to growth regulators of syconium tissues of each crop during development, explants were grown in culture media with and without growth regulators. The regulators used were 2,4-dichlophenoxy acetic acid, kinetin and gibberellic acid, each at two concentrations predetermined as suitable. After 4 weeks of *in vitro* growth microtome sections of these were observed for the evidence of types of histological growth. The wound response was formation of periderm, the extent of which was more in 2,4-D treatments. Some differences were observed in explant growth from two types of syconia at the same stage of development. These differences with their probable significance are described.

The King cultivar of fig (*Ficus carica* L.) produces two crops a year. The first-crop fruits are parthenocarpic and are borne on 1-year-old wood. They usually set at the end of April and mature at the end of June. Although fruitlets of parthenocarpic syconia lack embryos and endosperm, a series of supernumarary ovules develop over a period of 9 weeks or longer.[1] The endocarp of individual fruitlets sclerifies and imparts hardness to the so-called seed. Fruits of the second crop develop on current season's growth. They require caprification (fertilization) which occurs late in June and fruits mature at the end of August. Mature fruits of the first crop are generally larger and their form is more generally tapered from tip to the stem-end. The studies made on the shifts in levels of native harmones during the development of two types of syconia showed quantitative and qualitative differences in total 'free' auxins and acidic gibberellins.[2] Thus the differences exist in: (a) the environmental conditions under which the two crops develop; (b) the size and shape and in internal structure of syconia during their development; and (c) in levels of native harmones during their development. These differences suggested that relative sensitivity of the explants from parthenocarpic and nonparthenocarpic fruits to growth regulators in the culture medium might be different so far as their histological behaviour is concerned. This behaviour as reflected in the histological types of *in vitro* growth might give some clue to the *in vivo* growth of two types of syconia.

Materials and Methods

Measurements of Fruit Growth. The basal first-crop fig on previous season's growth and the basal second-crop fig on current-season's growth on each shoot were tagged for the determination of weekly growth increments. Horizontal diameter of 25 tagged fruits of each crop were measured with a vernier caliper.

Procedures for Culturing Fruit Tissue

Collection of Samples. Fig fruits of both crops were collected at 2-week intervals on the same calender dates for two years. Collections of the first year were used for preliminary tests of the suitability of the medium with and without added growth substances. Explants from ripening figs could not be cultured successfully. Samples were held in a refrigerator and were cultured within 1 week after collection.

Preparation of the Tissues. In preparation for culture, the surface of the fruit was sterilized with 0.3% sodium hypochlorite solution for 15 min followed by washing in sterile water. The fruits were then cut in halves, the outer surface pared, and the buds of flowers and the intervening epidermal wall removed aseptically. Small square segments were cut from the region of maximum diameter and each segment was weighed in a sterilized envelop made from weighing paper.

Composition of the Medium. The basic medium contained one-half the concentration of the micro- and macro-elements in Murashige and Skoog's[3] modified formulation of White's medium. Choline chloride (0.5 mg/l) was added to their list of vitamins and instead of 3, 2% sucrose was used. The source of iron was Fe-EDDHA [sodium ferric ethylene di-(*o*-hydroxyphenyl acetate)]. Bacto-agar (0.8%) was added to solidify the medium. The pH of the medium was adjusted to 6.2 before injection of 10 ml aliquots of medium into 15 × 150 mm test-tubes which were then autoclaved (20 min, 15 p.s.i., 120°).

KN (kinetin–6–furly amino purine) and 2,4-D (2,4-dichlorophenoxy acetic acid) were added to the basic medium beforeautoclaving. GA—gibberellin (K salt of gibberellic acid) was added to the test tubes of warm, unsolidified medium by filtering it through a millipore filter. The following concentrations were used: 0.1 μg/ml KN, 0.5 μg/ml 2,4-D and 10 μg/ml GA and also doubled concentrations of each. In addition all possible combinations of the growth regulators at higher concentrations, respectively, were tested. The basic medium without growth substances was used for control explants. Each weighed fruit segment was planted in a test tube containing 10 ml medium with or without growth substances. The tubes were plugged with cotton. Fifteen to twenty replicate cultured were grown for each test.

Preparation of Explant Sections for Histological Studies. After four weeks in culture, determinations of final weights of the explants were made and 2–3 representative explants from each treatment were fixed in Randolph's fixative. The explants were prepared for serial microtome sectioning and sections were stained in haematoxylene-fast green.

Results and Discussion

In vivo *Growth Pattern of Fig Syconia.* Growth curves for the first (parthenocarpic) and second (pollinated) crops based on diameter are presented in Fig. 1. Each point on the curve is the mean of 25 fruits. As the curves show the growth of the syconia of both crops proceeded in three stages, two stages of rapid growth separated by a period of slow growth, which were designated as periods I, II and III by Crane.[5] The first phase of rapid growth (period I) was due to cell division and some cell enlargement in the syconium wall, while the slow growth phase (period II) resulted from cell enlargement alone. The syconium reached its ultimate size by cell enlargement during the third phase (period III). The circles on the curves (Fig. 1) indicate time of sampling for explant culture in the growth media.

Histological Expression of in vitro *Growth of Fig Syconium.* Types of histological development in tissue explants of fig syconia during *in vitro* growth are shown in the photomicrographs (Figs. 2–8). In studies of growth, i.e. cell enlargement and expression of cell division, in explants subjected to different growth regulator treatments the growth patterns were like certain ones described by others[6,7] dealing with various plant materials. As might be expected, because of the impossibility of duplicating natural conditions for fruit growth when explants are grown in test tubes, none of the growth types found in explants duplicated that of syconium tissue *in vivo.* However, as a basis for comparison of cultured syconium segment with uncultured ones (Fig. 2), a brief histological description of the latter is pertinent.

A segment taken midway between apex and base of syconium wall shows similar structure in figs of both crops. The bulk of a syconium is formed of parenchyma, bounded by outer and inner epidermal layers. The latter is continuous with the epidermis of the pedicels which project from the inner surface of the syconium. Vascular strands and laticifers run through

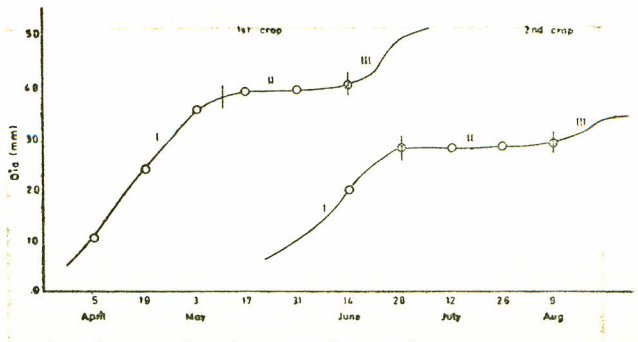

Fig. 1. Growth in diameter of figs of firsts (parthenocarpic) and second (pollinated) crops.

the parenchyma. Adjacent to both outer and inner epidermal layers, a few layers of compactly arranged isodiametric hypodermal cells are present. (Both epidermal and hypodermal layers were removed before explants were cultured). The parenchyma tissue in general is in the form of interlocking chains of cells surrounding air spaces which gradually increase in size as the cells enlarge during development. The largest cells and air spaces are formed midway between inner and outer epidermal layers. The cells surrounding the vascular strands and laticifers are more compactly arranged than cells of parenchyma farther from those structures. The smaller cells and nuclear sizes indicate that probable diploid condition and, therefore, greater possibility of mitotic induction than in the case of presumably polyploid nuclei of the larger parenchyma cells. (Evidences of polysomaty has been found in fig tissue by Bradley and Crane[1]).

Wound Response of Explants. One response to the wounding necessarily inflicted by excision of syconia segments was the formation of a periderm layer a few cells wide beneath the explant surface. Cells of that layer rapidly accumulated much tanning as a rule. Addition of 2,4-D to the medium increased periderm formation. The periderm appeared to greater or lesser extent whether explants were cultured on the basic medium or on media to which growth regulators had been added. In regions of the surface where it did not form, a few peripheral layers of cells deteriorated; they were apparently separated from the inner tissue by changes in the cell walls such as suberisation, which effectively prevented translocation to them as well as dessication of inner tissue.

Types of Growth in Control Explants. In control cultures from first crop figs, little or no mitotic activity took place and the small growth increments resulted entirely from cell enlargement. In controls from second crop figs, however, some cell multiplication occurred, originating primarily in the small cells around the vascular bundles. They were most numerous in explants of the June 14 and July 26 collections. Growth increment in those cultures amounted to more than 100% most of which was attributed to the cell enlargement, however.

Types of Growth in Explants on Culture Media Containing Growth Regulators. Cell Multiplication: Cell proliferation was initiated very close to cut surfaces (Figs. 4, 8 and 9) as well as in deeper regions of the explants (Figs. 3 and 5). In some cases evidence

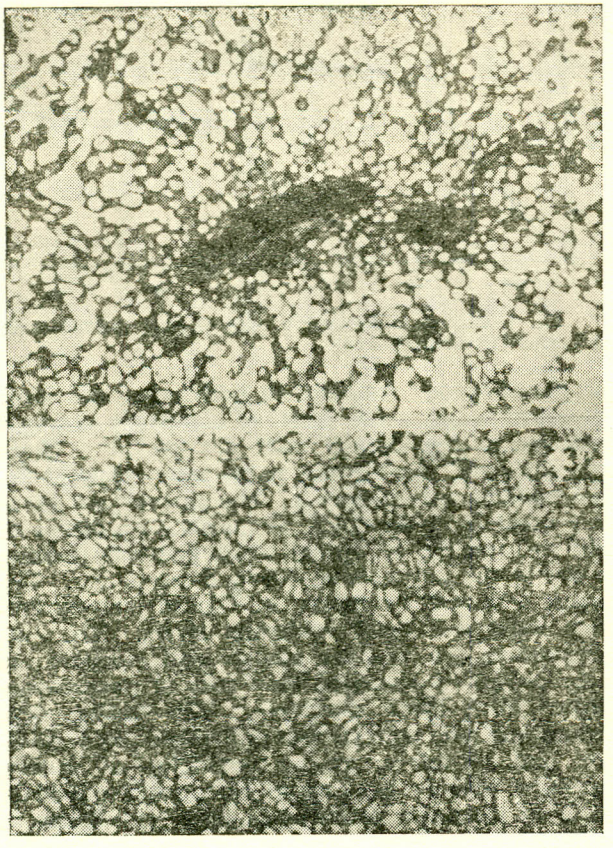

Fig. 2. Typical structure of tissue midway between epidermal layers of a mature syconium showing chains of cells around air spaces, and parts of vascular bundles (× 100)

Fig. 3. Structure of a typical well-developed callus tissue. In the centre of the central large-celled region are groups of vascular cells, but not clearly distinguished. Note evidence of a short cambium layer near upper right. The culture contained KN and 2,4-D. Darker regions are cells filled with tannins and frequently crushed by surrounding tissues × 100).

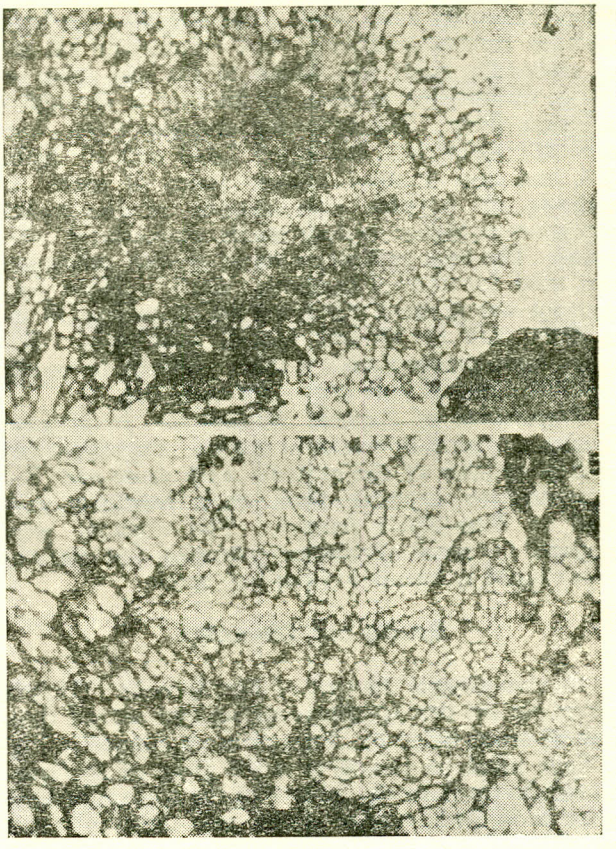

Fig. 4. Structure of a typical callus knob which was still growing at the time of fixation. Early formed cells on the periphery have become enlarged. Dark area shows crushed original syconium tissue which had accumulated tannins, as had also some regions of the callus × 100).

Fig. 5. Part of an extensive internal proliferation surrounded by original crushed explant cells. Internal callus showed similar structure to subsurface callus (Fig. 2), × 100.

of internal proliferation alone was found while in other signs of both subsurface and internal meristematic activity were seen. The proliferations originated from parenchyma around and within vascular strands, particularly in connection with vascular strands lying close and parallel to the explant surface exposed by cutting. The microscopic manifestation of proliferations, when they broke through the overlying cell layers, was the appearance of small callus knobs on explant surface. In some cultures such callus growth appeared when cultures were a week-old or less. Sometimes the callus developed to the extent of crushing adjacent tissue (Fig. 5).

The callus as seen in sections was distinguished from original syconium tissue by the relatively compact arrangement of its cells in contrast to the interlocking chains of parenchyma surrounding sizeable spaces, which is characteristic of the normal syconium tissue (Figs. 2 and 3). A typical highly developed and actively growing callus (Figs. 3–5) displayed considerable heterogeneity in the substructure contained and their determination. Cambial layers of limited span were still giving rise to small cells aligned in rows. The product of pervious cambial activity took the form of differentiated parenchyma of various sizes, still arranged in the linear series that gave evidence of their origin. In older regions of the callus were groups of relatively large parenchyma cells, which had presumably arisen from cambial layers formed early in callus development. Interspersed among these tissues were groups of as many as 12 small cells, oriented in various planes, each group having arisen relatively recently from a single cell as evidenced by the group still being contained within the original expanded cell wall. These groups were comparable to the 'pseudothalli' of Gautheret.[7] In the centre of such callus mass were scattered small groups of short and often distorted vessels and tracheids, sometimes accompanied by clusters of a few equally distorted phloem elements. Vessels and tracheids were reticulately pitted and the vessels had simple perforations. Callus masses having this relatively complex structure developed more extensively near the surface of the explants. At the other extreme of callus types were those in which development apparently had been of short duration and which were composed of somewhat enlarged parenchyma cells usually in serial arrangement, indicating their origin from previously active cambial

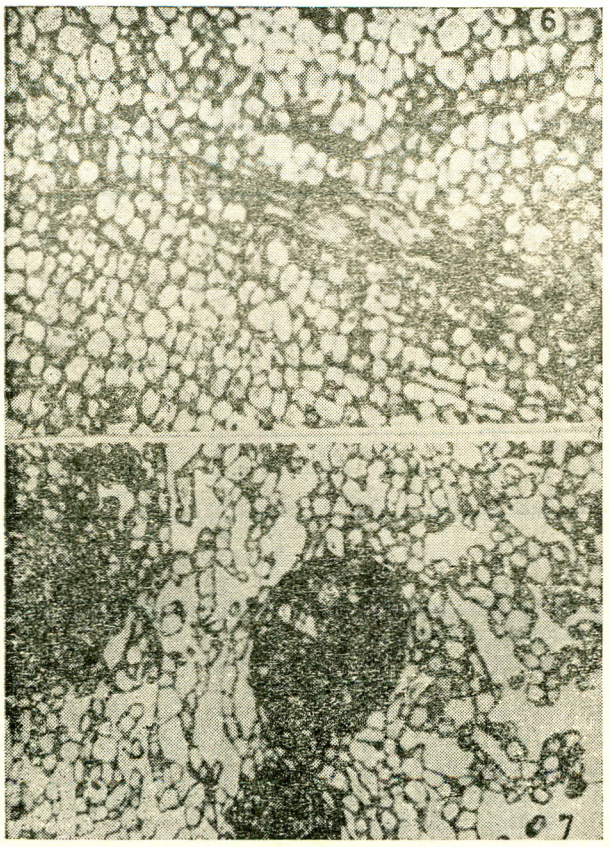

Fig. 6. Section of the explant showing limited internal proliferation, cells of which had differentiated into parenchyma. The proliferation apparently started in cells around the vascular bundle, part of which is shown. GA was added to the medium (× 100).

Fig. 7. Section of explant showing limited internal proliferation, some cells of which had differentiated into vessels and tracheids. KN and GA were added to the medium (× 100).

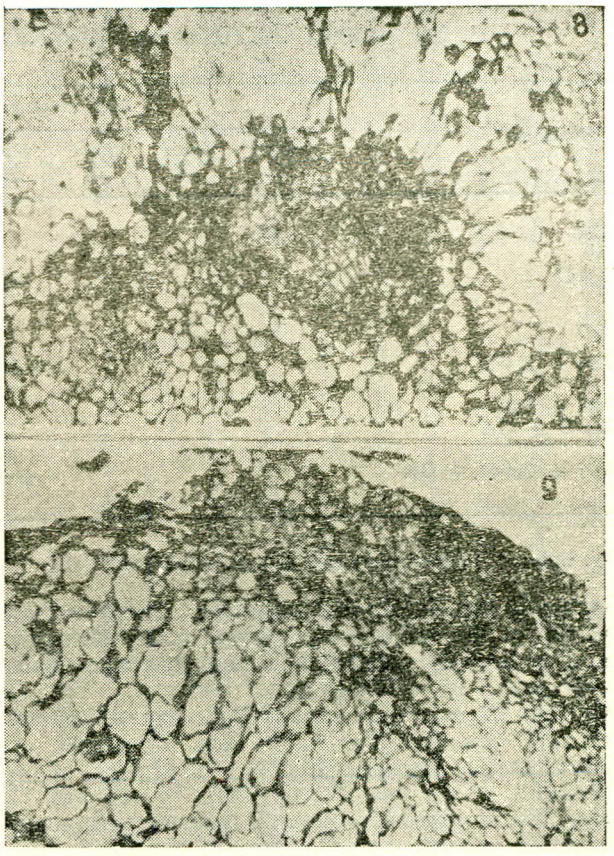

Fig. 8. Section showing a mass of newly formed callus originating from subsurface region of the explant. The extremely large cells near the surface represent the response of original syconium tissue to the KN and 2,4-D added to the culture medium × 100).

Fig. 9. A portion of an explant showing a proliferated mass of tissue from the surface region and greatly enlarged cells in the rest of the explant. KN, 2,4-D and GA were added to the medium (× 100).

layers (Fig. 6). The extent of differentiation and development of callus depended to some extent at least on the growth regulators added to the culture media as is discussed in the later section.

Cell Enlargement. All explants, whether treated with growth regulators or controls, and whether containing cell proliferations or not, included some regions in which cell enlargement had occurred. Explants from both crops at certain developmental stages grew mainly or entirely through cell expansion. Usually cells of the peripheral layers in some regions of the explants enlarged considerably. Addition of certain growth regulators to the media resulted in development of mammoth cells in some regions usually near explant surface (Fig. 8). The response to GA was particularly striking in this respect and to 2,4-D somewhat less so.

Histological Effects of Growth Regulators. The type of histological response to growth regulators, i.e. callus formation in addition to cell enlargement or the latter alone, varied from collection to collection (Table 1). Furthermore, the effect of certain growth regulators on explants from syconia at the same stage of development in some cases differed in two crops. KN induced internal callus growth only in certain samples of each crop. Proliferations were seen in explant sections from samples which responded well to KN alone, indicating interaction of added KN with endogenous auxins. In the first crop they occurred in three collections (April 5, May 31, and June 14), while in the second crop only in the last collection. Growth induced by 2,4-D alone was mainly through cell enlargement, particularly in samples during the period II of the second crop, where it stimulated the most growth. 2,4-D, however, induced relatively small amouts of subsurface as well as internal callus in collections during period II of the first crop (May 17 and 31) and in period I of the second crop (June 14 and 28). Addition of GA alone to the medium, while stimulating growth by cell enlargement in general, also stimulated limited internal cell divisions, the new cells having differentiated into parenchyma only. The cell division occurred in the first two samples of the first crop and in the last sample of each crop.

Both subsurface and inner proliferations were stimulated particularly by additions of both KN and 2,4-D to the medium. In the first crop they were limited to explants from period II but were less

TABLE 1. EXTENT OF CALLUS FORMATION IN EXPLANTS FROM PARTHENOCARPIC and POLLINATED FIG SYCONIA CULTURED ON MEDIA CONTAINING DIFFERENT GROWTH REGULATORS.

Treatment	Parthenocarpic						Pollinated				
	April 5	April 19	May 3	May 18	May 31	June 14	June 14	June 28	July 12	July 26	Aug 9
KN	+				+	+					+
2,4-D				+	+		+	+			
GA	+	+				+					+
KN + 2,4-D				+++	++	+	+++	+++	+++	+++	++
KN + GA							+	+			
2,4-D + GA						+					+
KN + 2,4-D + GA				++	++	+	++	++	++	++	+

+, little; ++, moderate; and +++, Abundant.

frequently seen in the last collection. Explants from syconia of the second crop showed callus formation in all collections treated with this growth regulator combination including those of period I. The addition of GA with KN and 2,4-D decreased the number and size of callus knobs and apparently enhanced cell enlargement (Fig. 9). In explants of two collections of the second crop (June 14 and July 12) where the combination of the 3 regulators stimulated maximum growth, some regions of giant cells were found. The combination of GA and KN stimulated cell enlargement primarily, except in the first 2 collections of the second crop, where the combination induced limited internal proliferations, similar to those caused by GA alone in the first two samples of the first crop. The combination of 2,4-D and GA also stimulated cell enlargement but in the last sample of both crops the combination induced internal proliferation which included differentiation of vascular elements.

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