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Development of the Foliaceous Cotyledons of Cucurbita Maxima

by

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Nelson
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DEVELOPMENT OF THE FOLIACEOUS COTYLEDONS OF CUCURBITA MAXIMA

HILMER C. NELSON

In many dicotyledons the seed leaves develop into foliar organs. The shift from storage structure to foliar leaf involves many changes and the morphological transformations in the larger cotyledons may be readily followed. This paper attempts to trace the changes in the cotyledons of the Hubbard Squash (*Cucurbita maxima*) through the germination period.

Cotyledons, in general, may be defined as the first leaves of the plant which do not arise from the primordial meristem. In spermatophytes as summarized by Goebel (5), the cotyledon may serve various purposes, namely (1) as a protective structure investing the bud, such as is found in Carex; (2) as a reservoir of reserve material, common in dicotyledons; (3) as a haustorium for the absorption of food for the embryo, common among grains; and (4) as a piston-like structure to push the seedling deeper into the soil during germination, such as is found in Phoenix. The above functions are in addition to that of photosynthesis which occurs in certain plants during the later stages of germination.

METHODS

For the anatomical study of cotyledons at various stages during their development sections of living and preserved material were used. Free hand sections were made of various portions of the cotyledon for general study. With the freezing microtome sections were cut parallel and at right angles to the surface. Permanent sections were prepared by the paraffin method in the usual manner. These were cut 10-15 μ in thickness and in the two directions noted above.

Of the various stains used the more satisfactory were an aqueous solution of malachite green and safranin with Delafield's haematoxylin. The former was used to stain temporary mounts prepared by the freezing method, the latter for permanent slides.

To determine the intervascular interval of the mature seed leaves as well as at earlier stages of development, an average of ten measurements was computed for each of five cotyledons and a final average of these was taken.

To obtain the rate of growth two sets of seedlings were marked and their cotyledons were measured; changes in the dimensions of one set were noted daily and of the other at three day intervals. Measurements of twenty cotyledons (ten plants) were taken at each reading.

In determining the various kinds of stored food present within the cotyledons in the resting seed Eckerson's microchemical tests for aleurone grains, starch and fats were used. The presence of starch was demonstrated by digestion with diastase and by microscopical examination of the cell contents.

HISTORICAL REVIEW

A number of investigators have studied the anatomy of the seed leaf and the changes occurring in embryonic tissues during germination. Hugo de Vries (10) working on clover investigated the external and internal anatomy of the seed as well as the modifications which take place in the different organs during germination. The changes occurring in the constitution of the embryonic tissues and perisperm during germination were followed by Gris (6), who also examined the modifications of the materials which these tissues enclose.

Godfrin (4) studied both tubercular (thick) and foliaceous (thin) cotyledons, making a morphological comparison of their anatomy, as well as the reserve food content of several plants including *Coulteria tinctoria*, Ricinus, Acer and others. He observed the following relations between the form of the cotyledon and the contents of its cells: (1) foliaceous cotyledons contain only aleurone; (2) tubercular cotyledons contain most frequently a mixture of starch and aleurone grains.

Pée-Laby (7) compared the seed leaves and true foliage leaves on the same plant in reference to their anatomical structure and found a marked similarity between them.

Auenmüller (1) investigated the structure of the cotyledons in the resting seed and the initial changes occurring in the germination of a number of pharmaceutically important plants. In his study of *Cucurbita Pepo* he found the cotyledon to consist of 22-24 cells in thickness and the palisade to be double-rowed, except above the procambial strand where a third row was present. He also found the seed leaf and radicle to be covered with a delicate cuticle in the resting seed.

In a developmental study of the true foliage leaves of Vitis and Catalpa Taylor (9) states that in these the intercellular spaces originate through the splitting apart of the cells because of unequal growth. The epidermal cells increased in size more than the contiguous palisade and spongy mesophyll cells and this inequality of growth separates the cells of the layers that expand less along the middle lamellae.

Duggar (3) found in experimenting with Canada field peas that the removal of the cotyledons after the second day of growth depresses the growth rate of the young plant. However, if the seed leaves are removed after seven days, the effect produced upon the rate of growth is very slight when the growth rate is compared with that of the control with cotyledons intact.

After an investigation of the nitrogen metabolism of Hubbard Squash seedlings, Reed (8) concluded that nitrogen compounds are necessary for the maintenance of the leaf, especially if it is exposed to intense illumination for long periods.

GROWTH CHANGES

The beginning of germination is manifested externally by the splitting of the seed coat at the micropylar end. The extrusion of the radicle occurs under favorable conditions after a two or three day period in the soil. This may vary with the rate of oxygen and moisture absorption by the seed coats.

After the radicle appears, growth is quite rapid. The peg grasps a portion of the seed coat by growing around it. The investments are further split apart along the edges, and as the hypocotyl arches up, the cotyledons are soon withdrawn from the seed coats.

Development of the root system keeps pace with that of the hypocotyl and cotyledons. The radicle elongates rapidly, and may achieve a length of approximately nine centimeters by the end of the fifth day. Lateral roots develop as the cotyledons are being withdrawn from the seed coats. Roots with the accompanying root hairs form an efficient anchoring and absorbing system early in seedling life.

EXPERIMENTAL

A number of experiments were carried out to determine the effects produced when parts of the young plants were removed.

EXPERIMENT 1

Marked seedlings grown in sand and supplied with a nutrient solution were divided into three groups. In one division (1) the seedlings were deprived of the plumule, in another (2) one cotyledon was removed and the third group (3) was allowed to develop undisturbed as a control. Measurements of the dimensions of the cotyledons were recorded at three day intervals.

Cotyledons of seedlings in which the plumules were removed experienced a longer period of growth and greater enlargement than the seed leaves in the other groups. It was interesting to note that in the second division the remaining cotyledon on each seedling attained a greater degree of enlargement than either of the seed leaves in the third group. Furthermore the early rate of growth was most rapid in the seed leaves of group 2.

EXPERIMENT 2

Another series of similar experiments was performed with seedlings grown in black soil and supplied with a nutrient solution. In these the plumules were removed from one group (1), the plumule and one cotyledon from each plant in another (2) and their growth compared with a third group (3) of normal seedlings. Measurements of the dimensions were recorded daily and are shown in tables 1, 2 and 3.

GROWTH RATES OF COTYLEDONS*

October 22 to November 27, 1927

Table 1

Growth Rate in Cotyledons with Plumule Removed

Days after wounding		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	36
Am't of growth over initial measurement	L	9	17	28	37	44	52	57	60	65	68	71	72	74	76	78	85
	W	6	9	15	19	23	27	29	30	32	32	33	34	35	35	35	37
Increments	L	9	8	11	9	7	8	5	3	5	3	3	1	2	2	2	7
	W	6	3	6	4	4	4	2	1	2	0	1	1	0	0	0	2

Table 2

Growth Rate in Cotyledons after the Removal of Plumule and One Cotyledon

Days after wounding		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	36
Am't of growth over initial measurement	L	13	21	34	44	51	60	64	69	73	76	78	80	81	83	83	91
	W	7	11	19	24	27	31	34	35	36	38	38	39	39	39	39	43
Increments	L	13	8	13	10	7	9	4	5	4	3	2	2	1	2	0	8
	W	7	4	8	5	3	4	3	1	1	2	0	1	0	0	0	4

Table 3

Growth Rate in Cotyledons of Normal Seedlings

Days		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	36
Am't of growth over initial measurement	L	12	18	22	25	27	30	30	32	33	35	35	35	35	36	36	36
	W	6	10	11	12	12	13	13	13	14	14	14	14	14	14	14	14
Increments	L	12	6	4	3	2	3	0	2	1	2	0	0	0	1	0	0
	W	6	4	1	1	0	1	0	0	1	0	0	0	0	0	0	0

The mutilated plants of the first and second groups developed their cotyledons to two to three times the dimensions of those on normal seedlings. It is of interest to note in this experiment that the cotyledonal growth curves of plants from which the plumules had been removed continue to rise, whereas that of the control seedlings soon straightens out, there being no further enlargement (text fig. 1). This interesting fact is confirmed by the data obtained in experiment 1.

EXPERIMENT 3

In order to determine the effect of cotyledon removal upon the plumule, both seed leaves were removed five days after sowing and at intervals of five days thereafter from successive sets of ten seedlings through a period of twenty days. At the end of the experiment a comparison of the surviving seedlings with normal seedlings was made as shown in figure 1.

A very striking degree of retardation in growth was noted in the initial set in which both cotyledons of each plant were removed at the end of the fifth day; a diminished retardation resulted in the remaining sets of seedlings until the removal of the seed leaves at the end of twenty days produced no visible effect upon the growth of the shoot. Of the seedlings whose cotyledons were removed at the end of the fifth day fifty percent perished.

DISCUSSION

The abnormal enlargement of the cotyledons following plumule removal indicates that the growth inducing agents which normally are translocated to the plumule remain within the cotyledons and stimulate the latter to greater development. Since normal develop-

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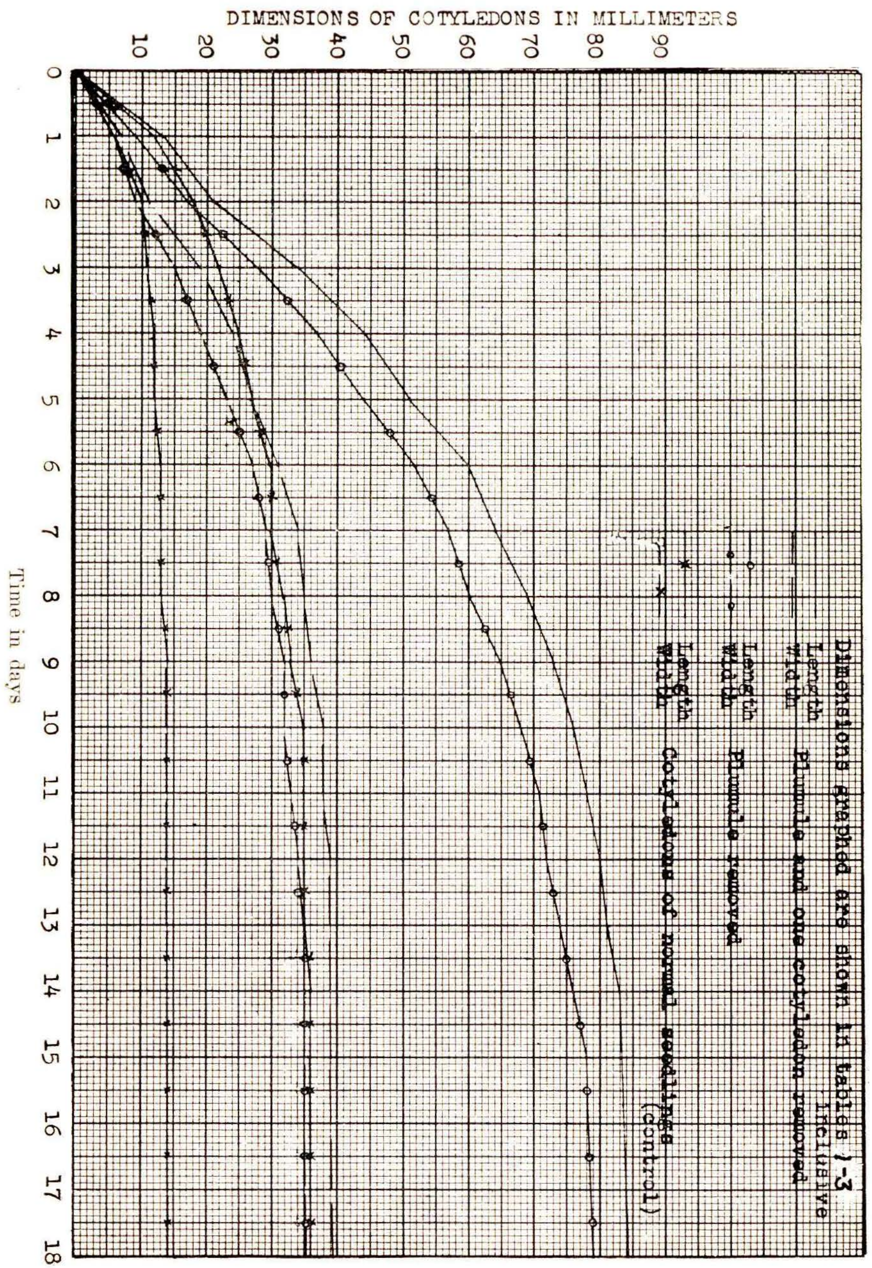
* Each record represents an average for ten plants with the exception of the final column which is the average for five plants. L = length in mm. W = width in mm.



TEXT FIG. 1. Growth curves of injured and uninjured seed leaves beginning on the fifth day of germination.

ment soon launches large foliage leaves, there is doubtless a correlation between plumule development and the limited size of cotyledons; in other words plumule development hinders cotyledon expansion.

In the light of Reid's experiments (8) it seems altogether probable that more nitrogen was made available to the cotyledons in the form of nitrogen compounds following the removal of the plumule and therefore a greater period of growth was experienced by the former.

The degree of retardation in seedlings deprived of their cotyledons is inversely related to the amount of photosynthetic tissue which was present when the mutilation occurred, for the plants exhibiting the greatest retardation possessed the smallest amount of photosynthetic tissue. The high rate of mortality in these seedlings was caused by a lack of adequate chlorophyll bearing tissue.

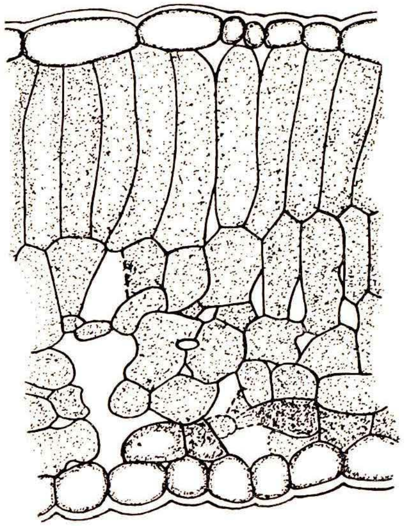
Retardation is due to the removal of food and other growth inducing agents stored within the cotyledon, for the earlier the cotyledons were removed, the smaller was the amount of photosynthetic and non-photosynthetic tissue which was produced by the injured seedlings.

MORPHOLOGY OF COTYLEDONS WITHIN THE RESTING SEED

An examination of the cotyledons of the resting seed revealed the presence of the same general regions as are found in the fully developed seed leaves and in the ordinary foliage leaves as well (text fig. 2, Pl. II, fig. 4 and Pl. III, fig. 9), but the regions are less distinctly differentiated within the resting seed than in the transformed cotyledon. The cell layers in the former are arranged in the following order: upper epidermis 1; palisade 2-3; spongy mesophyll 20; lower epidermis 1. The data refer to the number of cell layers in the central part of the seed leaf. The thickness decreases somewhat toward the edges of the cotyledon. The upper surface of the seed leaf is usually slightly concave and the lower surface is convex.

As indicated above the palisade region consists of two to three layers of cells (Pl. II, fig. 4) which are reduced at the edges to two cell layers. These cells are narrower than those of the spongy mesophyll and have their largest dimension at right angles to the upper epidermis.

The two regions, palisade and spongy mesophyll, merge imperceptibly into each other. In the later stages of development the line of demarcation becomes more distinct due to differences in the shape of the cells and to the compactness of the palisade as contrasted with the large air spaces found in the spongy mesophyll.



TEXT FIG. 2. Transverse section of the ordinary foliage leaf.

The cells of the spongy mesophyll region are initially arranged in more or less vertical rows at right angles to the epidermis, instead of the horizontal arrangement prevalent in the matured photosynthetic seed leaf in which the cells lie more or less parallel with the epidermal cells (Pl. III, fig. 9). In general, the cells of this region are larger than those of the palisade region.

There are present, even before germination begins, small air spaces forming angled openings of varying size at the corners of the mesophyll cells. These are significant in suggesting the method of further development of the air spaces which later become very prominent in the seed leaf.

The upper epidermal cells differ markedly from the lower in size and structure. The former are smaller than the latter and have thinner cell walls. There are no stomata present in either upper or lower epidermis of the cotyledon in the resting seed. An extremely thin cuticle extends over the outer walls of all epidermal cells. The principal procambial strands are usually nine in number, and there are no traces of lignification at this stage.

RESERVE FOODS OF RESTING SEEDS

The reserve food material in the resting seed was found to consist of aleurone, fat and starch. The aleurone grains are the most abundant food material within the cells at this stage, as reported by Barber (2) for *Cucurbita Pepo,* and are found in all cells of the cotyledon of the squash. Fats also form a large part of the food reserves and seem to be concentrated to a large extent in the epidermal cells. Starch is present apparently only in small quantities, for direct tests fail to reveal its presence. Nevertheless, the grains were visible in cells when they were highly magnified, and digestion with diastase gave evidence in support of this observation.

The food materials disappear first from the epidermal and procambial cells and last from the spongy mesophyll. Practically all of the reserves have been digested by the end of the fifth day following planting. At this stage the cotyledons are either emerging from the soil or have already assumed a horizontal position above the ground (Pl. I, figs. 2, 3). In either case photosynthesis has begun and as the photosynthetic activity increases, there is built up a reserve of starch in the seed leaves.

DEVELOPMENT AND DIFFERENTIATION OF CELL REGIONS

Stomata are present on the third day following planting. Their development is the same as that in the true foliage leaves and as the guard cells enlarge, an intercellular space forms beneath them.

The cuticle of the seed leaf remains quite thin until the tenth day of germination when local thickenings were observed bordering the guard cells at the stomatal openings. The presence of the cuticle was demonstrated by means of Sudan III (.5 gms. in 100 cc. of 70 per cent alcohol).

Trichomes make their appearance on the dorsal surface of the cotyledon during the second day after planting. At this stage the hairs may be protrusions consisting of one to three cells. These usually divide transversely resulting in the production of multicellular glandular or non glandular hairs. The former are stalked globular structures, whereas the latter are usually broad at the base and taper abruptly to slender pointed tips.

The lower epidermis shows the beginning of trichome development on the second day following that in the upper surface. At this time they are composed of only one or two cells. It may be significant to note that at this stage the cotyledons are being withdrawn from the seed coats (Pl. I, fig. 2), and that as long as the lower epidermis is in contact with the seed coat no development of epidermal hairs takes place. All trichomes are similar in their morphology and they occur most abundantly on the upper epidermis.

Changes in the size and form of the epidermal cells are marked and of considerable importance. These cells, examined twenty-four hours after planting, were apparently of the same size as those in the resting seed, but by the second day they averaged much smaller due to divisions in the peripheral cell layers. Cell multiplication proceeds so rapidly in the upper part of the cotyledons while the latter are still within the seed coats during the third and fourth days of growth, that the epidermal and adjacent palisade layers become depressed into furrows which extend into the spongy mesophyll of the seed leaf. No furrows were found in the lower side, although the cells in this region are also dividing. The dorsal grooves disappear during the fifth day as the cotyledons are being withdrawn from the seed coats.

The enlargement phase begins in the upper epidermis during the fifth and sixth days. It is evident at this time that some of the cells in this layer are undergoing division, whereas, others are enlarging.

All cell layers of the cotyledon have entered the enlargement phase by the seventh day of development. The lower epidermal cells are now slightly larger than those of the upper peripheral layer, and it is at this time that the wavy lateral walls characteristic of lower epidermal cells appear.

A steady increase in the size of cells of the peripheral layers continues from the seventh to the twentieth days of germination, with the cells of the lower epidermis continuing somewhat larger than those of the upper layer. Examination of sections at the thirty day stage showed no further enlargement in these layers over the twenty day stage.

The first day's growth reveals little change in the palisade of the cotyledon. Sections of cotyledons which have remained for two days in the soil show that the cells in this region are noticeably narrower than those in the corresponding part of the resting seed and have apparently begun cell division which continues through the third, fourth and fifth days of growth (Pl. II, figs. 5, 6). Cell enlargement begins in the palisade during the seventh day of development and continues for two weeks (Pl. III, figs. 7, 8).

The most conspicuous changes in the development of the seed leaves are in the region of the spongy mesophyll. These cells apparently remain undivided throughout the transformation of the cotyledon into the foliar organ despite the active cell division and enlargement occurring in the other regions of the seed leaf. Extensive changes occur, however, not only in the amount of air space in this region, but also in the volume and form of the cells.

Transverse and horizontal (cut parallel to the epidermis) sections of the dormant cotyledon show small angular spaces at the corners of the cells in the palisade and spongy mesophyll regions. Sections of cotyledons one day after planting show no variations, but one day later a slight increase was noted in the degree of air space development at the corners of the cells. This change continues during the third and fourth days of growth and by the end of the fifth day an extensive system of air spaces is present (Pl. IV, fig. 10).

By the seventh day many of the mesophyll cells have undergone a change in relative position so that some are elongated parallel with the epidermis, while others still remain at right angles to the peripheral layers. The volume of these cells has become considerably enlarged, and many show a distinct lobing at the corners.

In the final arrangement most of the spongy mesophyll cells have either become spherical or have elongated in the dimension parallel with the peripheral layers (Pl. III, fig. 9). While the epidermal and palisade cells have undergone a period of division followed by enlargement, the spongy mesophyll cells have enlargement without mitoses. Air spaces have become much more extensive in the spongy mesophyll than in the palisade region. Apparently the epidermal cells have increased in size and number more rapidly than the mesophyll cells, and this inequality of growth has pulled apart the cells which enlarged less. This development is similar to that of the true foliage leaves of Vitis and Catalpa as reported by Taylor (9).

The same spreading venation is found in the seed leaf as occurs in the true leaf with this difference: that the veins of the cotyledons do not unite at the base, but extend separately into the petiole and stem. There are seven major veins in the cotyledon which vary considerably in length and degree of development. From these numerous lateral veins branch forming a reticulate network in the lamina of the seed leaf.

The vascular regions (procambial strands) in the resting seeds are filled with stored food. The cell walls are quite thin with no traces of thickenings present in the walls of the cells destined to become the vessels. Increase in thickness of walls begins promptly, for in seeds that had been soaked in water for seventeen hours and placed in the soil for twenty-four hours, a few less dense cells in the procambal strand were found with thickened walls. Seed leaves one day later showed a few well developed vessels in the principal vascular strands. The central strands also contained a large number of cells emptied of their food reserve. Vascular bundles three days after planting revealed the presence of more than a dozen thick-walled vessels; the remaining cells in the bundle, emptied of their food reserve, were undergoing differentiation into their respective tissues.

The cotyledon possesses an efficient minor venation. There are two systems of these veins (1) those that ramify through the lower part of the spongy mesophyll, which are well developed and supported by mechanical cells and (2) those that spread through the upper part of this region directly beneath the palisade layer, which are very simple and more abundant. The intervascular interval between these palisade veinlets becomes greater as the seed leaf increases in size, but is less in the mature cotyledon than in the foliage leaf.

The intervascular intervals of palisade veinlets (second system of minor veins) of cotyledons and foliage leaf measured from the edge of one veinlet to that of the other are as follows: cotyledon of dormant seed .064 mm.; seed leaf after five days growth .084 mm.; average of mature cotyledons aged 40, 43, 37, 82 and 40 days .136 mm.; foliage leaf .146 mm. Each of the preceding records represents an average of fifty measurements in five cotyledons, and the same number in the foliage leaf.

DISCUSSION

The palisade, spongy mesophyll, and epidermal tissues of the cotyledons are first specialized for food storage and are not distinctly differentiated within the resting seed. During germination changes in the morphology of these cells accompany changes in function enabling the transformed seed leaves to perform efficiently the work of a foliage leaf. The large quantity of protein stored in the cells of the resting seed makes possible the rapid development of the seedling. The evidence indicates that the small angular spaces at the corners of the mesophyll cells within the cotyledons of the resting seeds are formed by the separation of the cell walls along their middle lamellae. The subsequent enlargement of these intercellular spaces produces the extensive system of air spaces adjoining the mesophyll cells of the transformed cotyledon.

The temporary furrowing of the upper surface of the cotyledon is probably caused by a greater proliferation of cells in the upper epidermis and palisade region than in the mesophyll and lower epidermal layers. Unable to expand laterally due to the constricting effect of the seed coats, these tissues were forced downward compressing the spongy mesophyll which at this time was being rapidly exhausted of its food reserve. It seems probable that the force exerted by the dividing and enlarging cells of the peripheral layers directed against the edges of the seed coats caused a further extension of the splitting already begun at the point where the radicle and hypocotyl had emerged.

Sections show that coincident with the expansion of the cotyledon in the initial phases of germination, the epidermal and palisade cells undergo repeated division and enlargement as contrasted with a less degree of enlargement in the spongy mesophyll, causing the cells in the latter region to be subjected to lateral tension.

The separation of the spongy mesophyll cells takes place along the middle lamella and results in the production of a large number of air spaces. It seems probable also that since the epidermal cells divide and enlarge more rapidly than the contiguous mesophyll, this unequal growth causes air spaces to develop in the palisade region. These changes resulting in an extensive system of air spaces are essentially like those described by Taylor (9) as occurring in the development of the true foliage leaves of Vitis and Catalpa.

The intercellular spaces formed during germination are merely enlargements of the small angular spaces at the corners of the cells in the resting seed and seem to have resulted from tensions due to unequal growth of the several cell layers.

Vascular differentiation may begin very early after the planting of the seed, as shown by the launching of vessels in the chief procambial strands of the cotyledon within twenty-four hours. These vessels are undeveloped in the resting seed and therefore their appearance is associated with the beginning of seedling development.

The differentiation of the conducting tissues keeps pace with the increase in the size of the cotyledon. The intervascular interval is less in the developing than in the mature seed leaf, making possible rapid translocation of food reserves during the critical period of germination.

Since the intervascular interval of the seed leaf is less than that of the true leaf, translocation of materials may occur more speedily in the cotyledon than in the true foliage leaf.

SUMMARY AND CONCLUSIONS

1. The cotyledons of *Cucurbita maxima* in the resting seed contain the same general regions as are present in the mature foliar cotyledon.
2. Stomatal development occurs in the same manner as in the ordinary foliage leaf.
3. A thin cuticle is present in the resting seed as a delicate covering over the cotyledons.
4. Trichome differentiation begins early in the process of germination and starts first on the upper epidermis.
5. Germination begins within twenty-four hours after planting and may continue for twenty days, or until the supply of growth inducing substances within the cotyledons is exhausted.
6. Cell division begins as early as the second day of development and continues through the fifth day.
7. Cell division predominates in the epidermal layers and palisade zone of the seed leaf from the second to the fifth day of germination, after which cell enlargement begins and continues until the seed leaf becomes mature.
8. Cells in the epidermal and palisade layers divide and enlarge more actively than in the associated spongy mesophyll region. The lateral expansion of the cotyledon seems to be due primarily to the division and enlargement of epidermal cells.
9. The organization of the spongy mesophyll is doubtless aided by tensions, due to the unequal growth of this zone compared with the adjacent layers.
10. The spongy mesophyll cells seldom divide, enlarge early, become spherical and then elongate laterally parallel to the epidermis. The greatest development of the intercellular space occurs in this region.
11. Growth in area is distributed uniformly throughout the seed leaf.
12. There is a definite correlation between plumule development and the size of cotyledons.
13. The presence of normal cotyledons on the seedling during germination is essential for the unimpeded development of the plumule.

14. The cotyledon possesses two general groups of minor veins, one in the palisade and the other in the spongy mesophyll.

15. The intervascular interval between the palisade veinlets becomes greater as the cotyledon increases in size, but is less in the mature foliar cotyledon than that between the veinlets in the true foliage leaf.

16. Normal development of the cotyledon to maturity from the seed condition probably covers a period of about twenty days. This period may be lengthened to approximately thirty days by the removal of the plumule immediately after the appearance of the cotyledons above the soil during germination.

17. The intercellular spaces result from a separation of the walls of the mesophyll cells along the middle lamella.

This report is the result of a two years' study of the morphology of cotyledons under the direction of Professor R. B. Wylie, Head of the Department of Botany of the State University Iowa, to whom the writer is deeply grateful for helpful advice and criticism and for his suggestion of this problem.

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EXPLANATION OF PLATES

PLATE I

Fig. 1. Photograph of seedlings showing the effects of cotyledon removal upon their development: *a-f* showing retardation caused by the removal of seed leaves five days after planting; *g-i* exhibiting delayed development following the removal of cotyledons ten days after planting; *j, k,* showing retarding effect after the removal of the cotyledons fifteen days following planting; *l, m,* showing no effect after the removal of cotyledons on the twentieth day; *n, o,* normal seedlings with seed leaves intact. Age of all plants is twenty-six days.

Figs. 2-3. Successive stages in the development of squash seedlings.

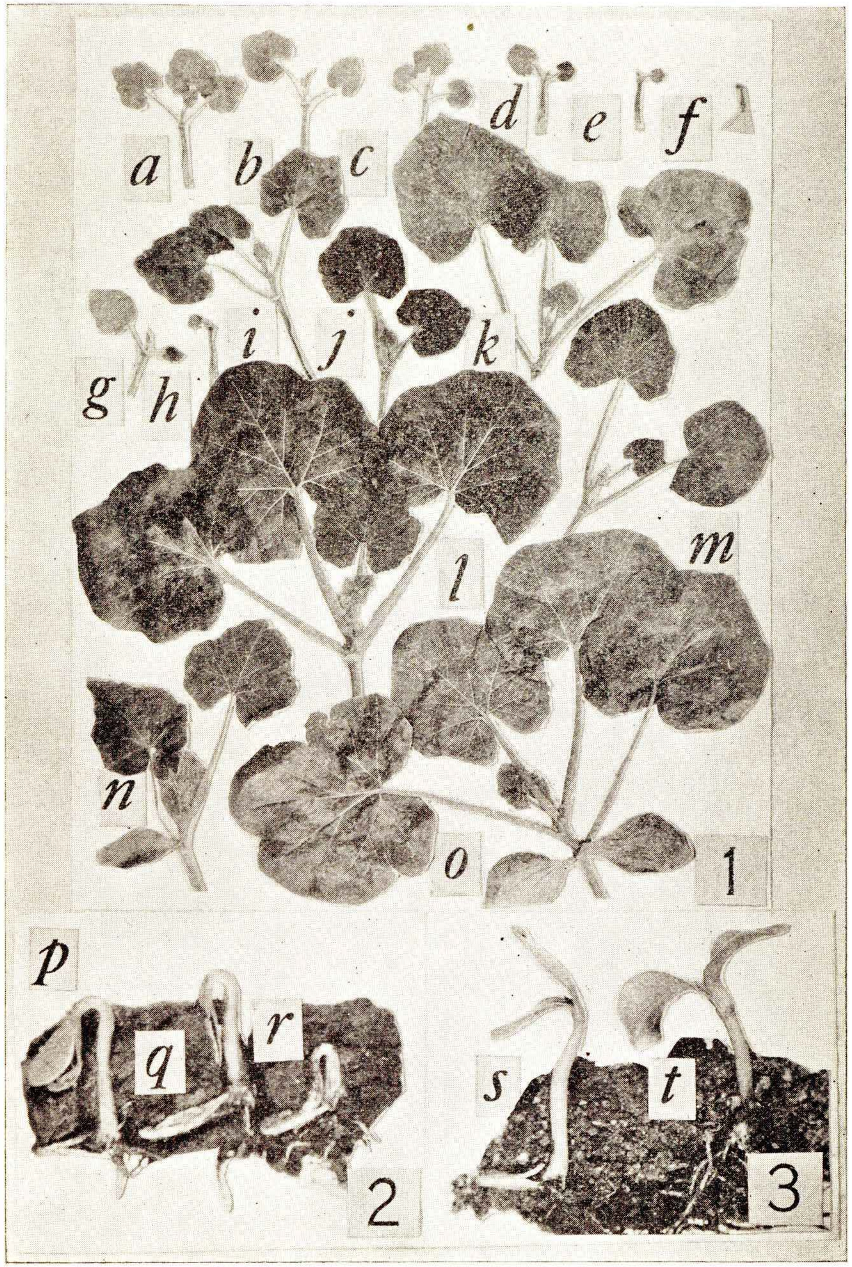


PLATE II

Fig. 4. Transverse section of cotyledon in dormant seed condition. x154.

Fig. 5. Transverse section of cotyledon after a five day period in the soil showing large air spaces in spongy mesophyll. x154.

Fig. 6. Transverse section of seed leaf after soaking in water for twelve hours and one day's sojourn in the soil. x154.

PLATE II

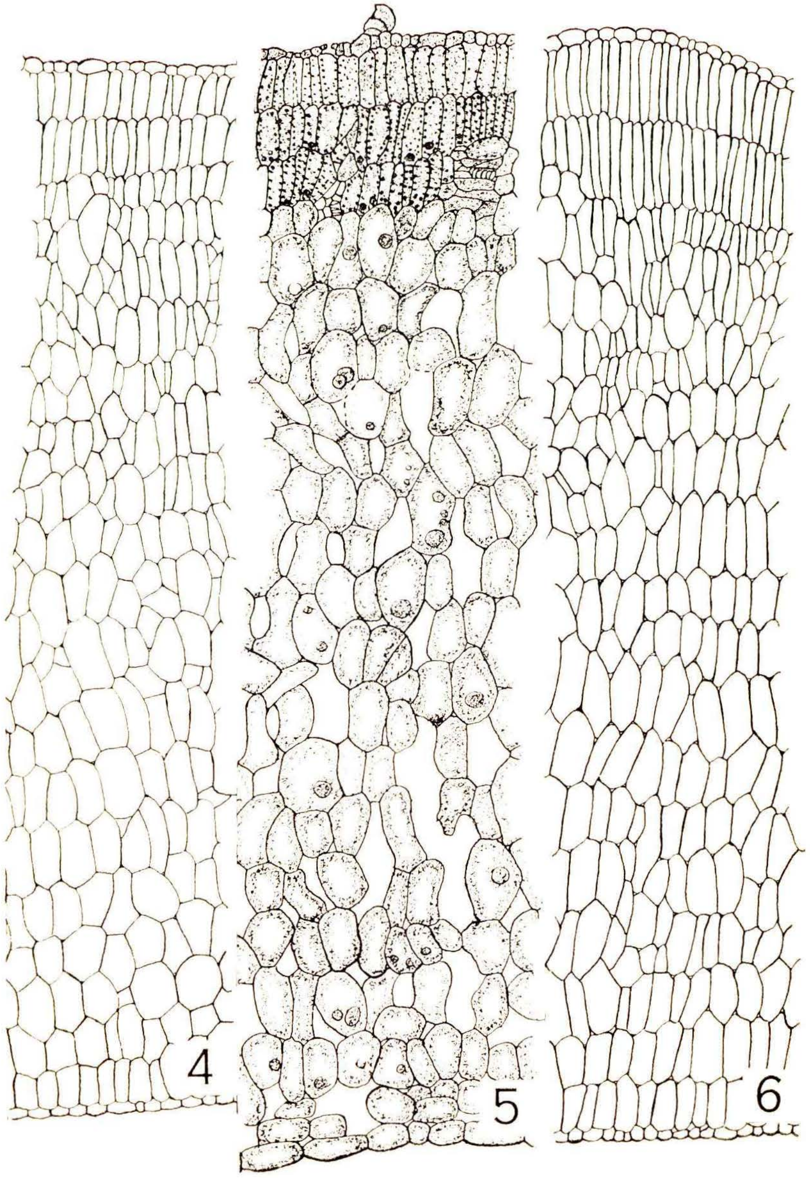


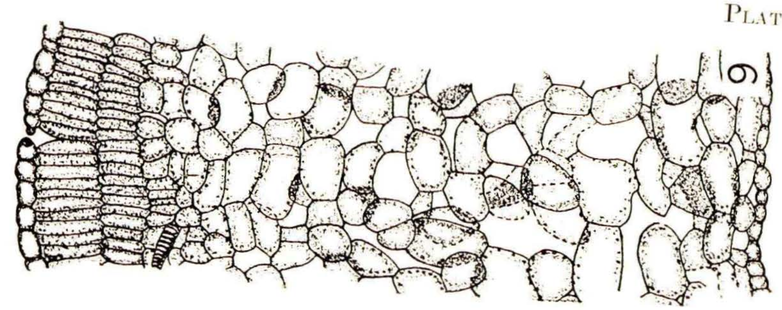
PLATE III

Fig. 7. Transverse section of seed leaf showing extent of development on the seventh day of growth. x154.

Fig. 8. Transverse section of cotyledon fourteen days after planting. x154.

Fig. 9. Transverse section of seed leaf on the thirtieth day showing transformed spongy mesophyll cells. x154.

PLATE III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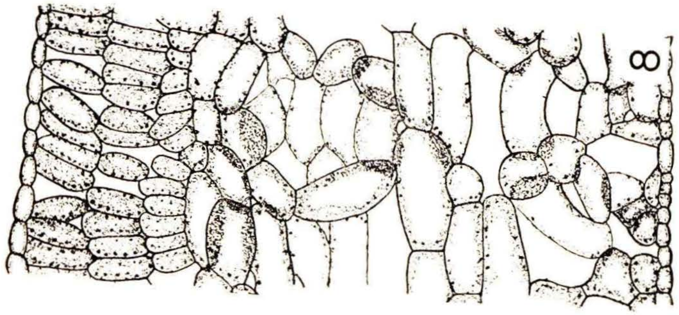
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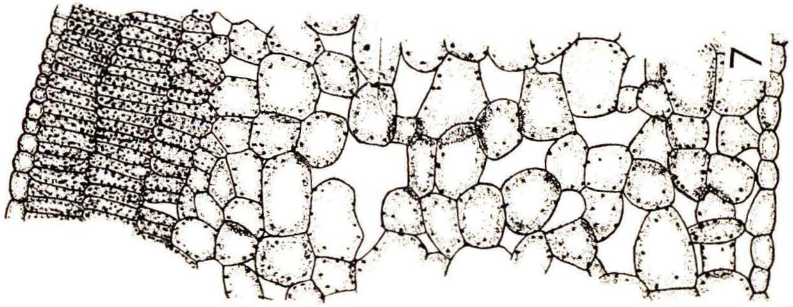
7

PLATE IV

Fig. 10. Sections cut parallel to the peripheral layers showing structure of seed leaf five days after planting: *a*, showing cell enlargement and stomatal development in upper epidermis; *b*, *c*, showing reserve food within the cells and development of air spaces in upper and middle portions of the palisade region; *d*, *e*, showing reserve food within the cells and development of air spaces in middle and lower portions of the spongy mesophyll.

All drawings were made with the aid of a camera lucida and a Zeiss apochromatic objective 90 n. a. 1.30 and ocular 10x.

PLATE IV