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Characteristics of Hook Formation by Bean Seedlings

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BERNARD RUBINSTEIN[1]
Michigan State University-Atomic Energy Commission, Plant Research Laboratory, Michigan State University East Lansing, Michigan 48823

ABSTRACT

Explants were isolated from 6-day-old etiolated bean seedlings (*Phaseolus vulgaris* L. cv. Black Valentine) containing the cotyledons with 4 mm of hypocotyl just below the node and/or the epicotyl. During incubation on distilled water, uneven growth of the hypocotyl or epicotyl occurred resulting in the formation of a hook. The more rapid growth of the side which became convex was not dependent upon the presence of the slower growing concave side. It was concluded that the main axis has an intrinsic capacity for asymmetric growth. The growth leading to hook formation was inhibited by α-naphthaleneacetic acid at concentrations above 0.2 milligram per liter.

When a bean seedling is under the soil or in the dark, the apical portion of the hypocotyl is bent downwards almost parallel to the basal portion, forming the so-called hypocotyl hook. This configuration is widespread among dicotyledonous plants and probably protects the tender apex from injury while breaking through the soil. If the seedling continues to grow in the dark, the concave (or inner) portion of the hypocotyl elongates at the base of the elbow. Elongation at this location would result in hook opening, but an accelerated growth of the concave (or outer) surface just above the existing hook occurs simultaneously, thereby preserving the hook (10). Hook opening and hook formation are thus two continuous, closely synchronized processes which result, under etiolated conditions, in the apparent movement of the hook up the hypocotyl, past the cotyledons, and into the epiocotyl.

I have examined the processes leading only to formation of the hook by isolating and culturing tissue located above the hook which had not yet become part of the curved area. Such experiments were designed to determine if the asymmetric growth of the upper portion of the seedling leading to hook formation is controlled by the curved tissue of the elbow, or whether this growth is an inherent characteristic of the tissue itself. Darwin (1) has already concluded after observing the germination of *Vicia faba* seeds imbedded on pins at various angles that formation of the hook was not due to a mechanical resistance exerted by the medium or to geotropic orientation.

MATERIALS AND METHODS

Phaseolus vulgaris L. (cv. Black Valentine) was grown at 25 C in the dark on vermiculite and watered with tap water at time of sowing and after 4 days. Hypocotyls from 6-day-old plants were selected which were straight from the cotyledonary node to a point about 8 mm below. In most older plants, the curved tissue comprising the hook extended to the cotyledons. The apical portion of the plant was then removed to include the cotyledons and all tissue distal to them as well as 4 mm of the hypocotyl just below the cotyledonary node (see upper portion of Fig. 1). The seed coat, if still attached, was removed, and the explants placed in 90-mm Petri dishes containing a filter paper disc and 10 ml of distilled water. Compounds to be tested were added to the water.

The Petri dishes were then incubated at 25 C in either darkness or continuous red light for 2 or 3 days. The light source was one Ken-Rad fluorescent tube wrapped in three layers of red cellophane and filtered further by Rohm and Haas No. 2224 red Plexiglas. All manipulations were carried out under a dim green safelight which met the specifications of Withrow and Price (13).

Measurements of hook formation by explants were made by shadowgraphing the material and determining the size of the angle formed by one line perpendicular to the cotyledonary node, and another line parallel to the lower 2 mm of the hypocotyl. In order to provide an expression of hook formation, this angle was then subtracted from 180° which was the starting angle of a completely straight section. Thus, the larger the numerical value of the angle, the more pronounced the hook formation. Growth was determined from shadowgraphs by measuring length along the middle of the hypocotyl from the cotyledonary node to the basal cut surface.

The starting material for culturing apical tissues was obtained by removing from a 4-day-old etiolated plant the primary leaves along with the apex above and about 2 to 3 mm of epicotyl below (see left half of Fig. 3). The explants were sterilized in 1% (v/v) hypochlorite, rinsed in distilled water, and imbedded in 0.9% (w/v) agar containing the basal medium of Miller (8). Kinetin at 3 μM and IAA at 10 μM were also added. All manipulations were performed under the green safelight and explants were incubated in the dark for 20 days.

RESULTS AND DISCUSSION

Studies of hook formation were conducted using only tissue located above the existing curved region of the hypocotyl (upper half of Fig. 1). It can be seen in the lower half of Figure 1 that after 2 days in the dark, the hypocotyl portion of the explant doubled in length (as measured by the distance from the cotyledonary node to the proximal cut surface down the middle of the hypocotyl) and growth was distributed unevenly so that a curvature resulted. If an ink mark were made on the cotyledons at the time of isolation of the explant, it was possible to show in every case that the side which would have become the concave side if still attached to the plant, also became the concave side in the explant. Once formed, the

[1] Present address: Department of Botany, University of Massachusetts, Amherst, Mass. 01002.

hook moved past the cotyledons into the epicotyl (see right half of Fig. 2).

The effects of various parts of the explant on hypocotyl curvature and growth are shown in Table I. It was quite clear that removal of the apex or all of the tissue above the cotyledonary node had no influence on either hook formation or over-all increase in length. The absence of the cotyledons, however, greatly reduced both processes. The requirement for cotyledons seems similar to that shown by Katsumi *et al.* (4) for hypocotyl growth in cucumbers.

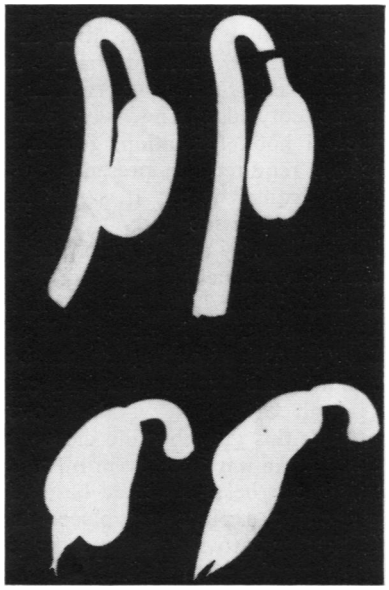

FIG. 1. Upper half shows the upper portion of a 6-day-old bean plant (left) and the explant used (right). Lower portion shows growth and curvature of the hypocotyls of two explants after 2 days incubation on distilled water.

Table I. *Influence of Apex, Epicotyl Internode, and Cotyledons on Hypocotyl Growth and Curvature*

The intact explant consisted of the upper 4 mm of hypocotyl, the cotyledons, and epicotyl. Ten explants were incubated in the dark on distilled water in Petri dishes for 72 hr. Curvature was measured as a change from a starting angle of 180°. Growth is the distance from the cotyledonary node to the basal cut surface minus the initial length of 4 mm. Data expressed as the mean ± SE.

Explant	Hook Formation	Growth Increase
	degrees	*mm*
Intact	126 ± 23	9.8 ± 1.0
Minus apex	133 ± 9	11.2 ± 1.2
Minus apex and epicotyl internode	149 ± 17	11.2 ± 1.5
Minus cotyledons	39 ± 13	2.6 ± 0.7

The asymmetry of growth of the hypocotyl was examined further by isolating and measuring the growth of only that area which was to become either the concave or convex surface. This was done by removing the inner or outer two-thirds of the hypocotyl up to the cotyledonary node and then measuring the straight growth of the remaining portion. The data for the explants in Table II compare the growth of the hypocotyl sections with the curvature of whole hypocotyls in dark or continuous red light. After 3 days of incubation, hook formation by explants with intact hypocotyls was only 59° from the original in the light compared to 138° curvature by hypocotyls in the dark. Growth of the isolated outer or inner sections of the hypocotyl reflected to a large extent the amount of curvature in the explants with intact hypocotyls. In the dark, the isolated outer one-third of the hypocotyl grew significantly more than the inner one-third. In the light, inner and outer hypocotyl sections grew to approximately

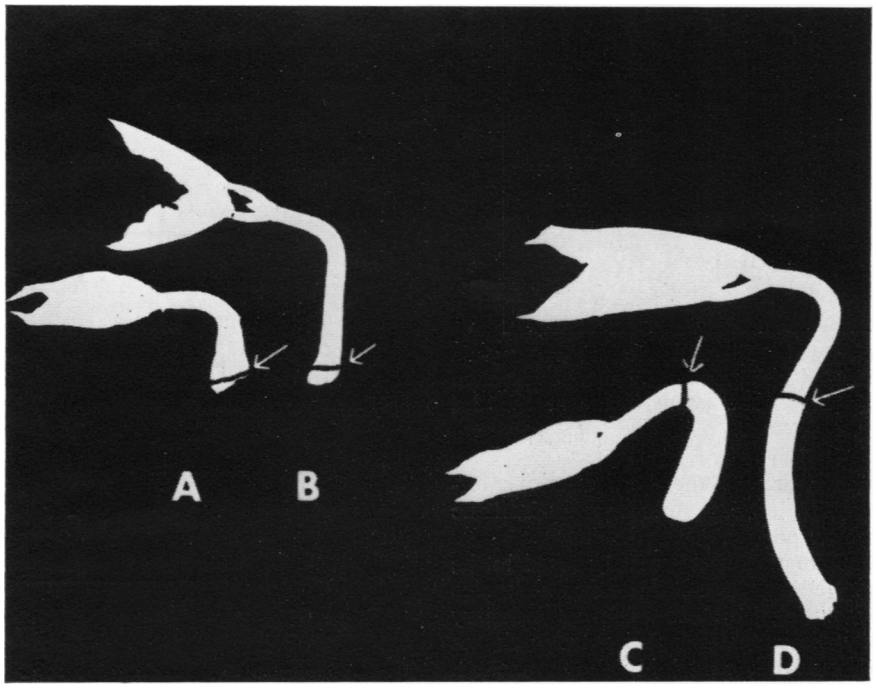

FIG. 2. Growth after 5 days of explants taken from the apical portion of etiolated bean seedlings in which 4 mm of hypocotyl was cut away or left intact. A: Hypocotyl removed and incubated in the dark; B: hypocotyl removed and incubated in continuous red light; C: 4 mm hypocotyl intact and incubated in the dark; D: 4 mm hypocotyl intact and incubated in continuous red light. Cotyledons were removed just prior to shadowgraphing. Arrows point to location of cotyledonary node (lines drawn in).

Table II. *Comparisons between Curvature of Intact Hypocotyls and Growth of the Isolated Inner or Outer Portion*

Ten explants consisting of epicotyl, cotyledons, and the apical 4 mm of hypocotyl were incubated in distilled water for 72 hr. Using similar explants, two-thirds of the hypocotyl which would become either the convex (outer) or concave (inner) side was removed with a scalpel up to the cotyledonary node. Straight growth was measured on the portion of hypocotyl remaining; the initial length was subtracted to provide the data for growth increase. Curvature was measured using only explants with hypocotyls intact. Data expressed as mean ± SE.

	Hook Formation	Growth Increase	
		Outer one-third	Inner one-third
	degrees	*mm*	
Dark	138 ± 7	5.8 ± 1.3	3.3 ± 0.7
Continuous red light	59 ± 20	6.4 ± 1.5	7.3 ± 1.7

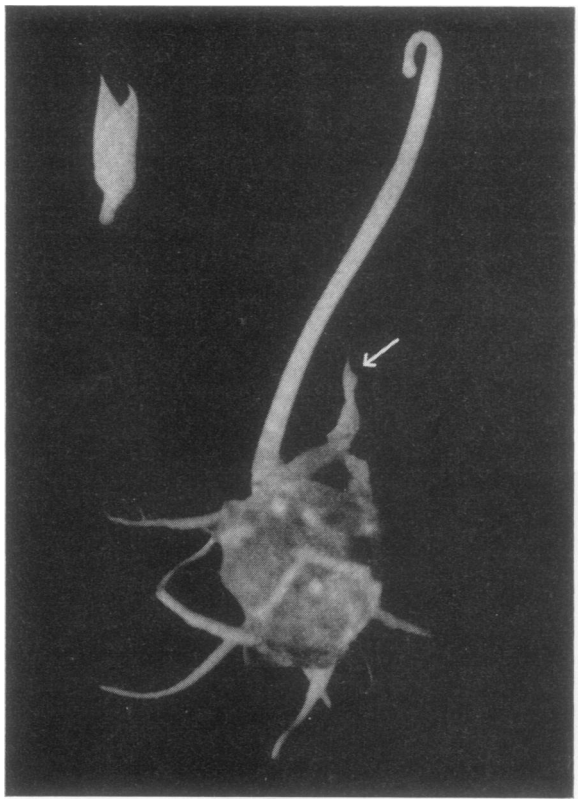

FIG. 3. Hook formation by the apical portion of a 4-day-old etiolated bean plant (left) which was cultured on nutrient medium for 20 days in the dark (right). Arrow points to the unexpanded primary leaf.

the same length. One can conclude, therefore, that the differential growth rates leading to curvature are an intrinsic property of each half of the hypocotyl.

Up to this point, hook formation had been measured in the tissue just below the cotyledons. Hook formation was also observed in epicotyls by cutting just below the cotyledonary node and incubating only the cotyledons and epicotyl tissues as done previously with explants containing the hypocotyl. Figure 2, A and B, shows that asymmetric growth of the epicotyl resulted in a hook of about 90° by the 5th day. Epicotyl tissue grew more rapidly in continuous red light, but a hook was evident here, too. Hook formation in hypocotyl tissue after 5 days in dark or light is shown for comparison in Figure 2, C and D.

It should be pointed out that in isolated explants not only the asymmetry of growth between inner and outer halves is similar to that occurring on the intact plant, but the timing of growth as well. For example, explants with hypocotyls produced a hook by the 3rd day, and it moved into the epicotyl by about the 5th or 6th day (Fig. 2, C and D). Explants without the hypocotyl showed little growth at first, and it was not until about the 5th day that the epicotyl hook was clearly present (Fig. 2, A and B). Intact, etiolated plants also had the hook in the epicotyl at this time. Thus, control of growth asymmetry as well as time of growth resides in the tissue itself and seems not to be regulated by other organs of the plant.

The role of the cotyledons, however, remained unclear; similar to hypocotyl hook formation, growth and hook formation by epicotyls required the presence of the cotyledons when incubated in distilled water. It is possible, however, to observe the most apical tissue without the influence of the hypocotyl and cotyledons by removing just that portion of the epicotyl above the cotyledons and culturing it on a nutrient medium in the dark (left half of Fig. 3). After 14 days of culture, a hook was observed in about half of the explants, and after 20 days (right half of Fig. 3), the basal portion of most of the explants had proliferated into callus, roots had formed, and growth of the internode above the primary leaves had occurred; this growth quite clearly resulted in the formation of a hook. An unexpanded primary leaf attached to an enlarged petiole can be seen at the base of the internode (arrow). When such an explant was placed in the light, the hook straightened after 3 to 5 days, and the leaf next to the apex expanded to form the first trifoliate.

It appears, then, that tissue all along the main axis above the existing hook has an intrinsic capacity for asymmetric growth leading to hook formation. This capacity is probably

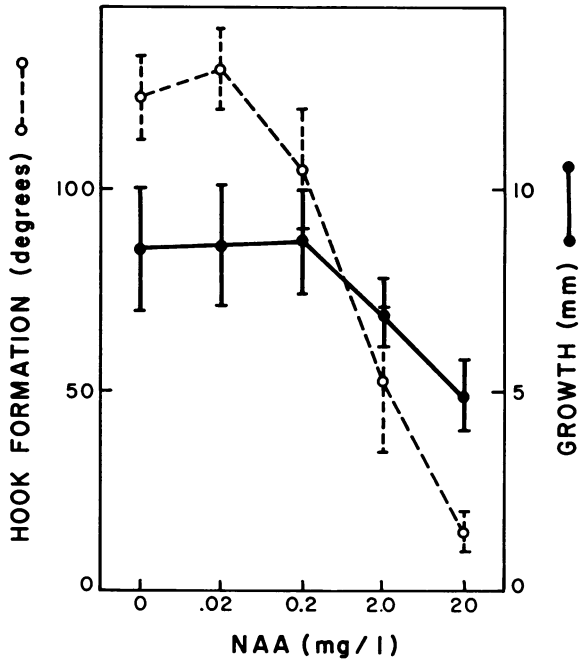

FIG. 4. Effect of α-naphthaleneacetic acid on the development of curvature and increase in growth of the hypocotyl portion of explants incubated for 72 hr in the dark. Vertical lines represent the standard error of the mean for 10 explants.

established early in the development of the seed; it has already been reported that embryos of bean seeds which were 18 mm long and reached only 50% of their maximum fresh weight formed normal hooks (12).

It may still be possible that curvature is induced by unequal transport of a substance out of the cotyledons. But the insertion of the cotyledons is parallel to the plane of the hook (see Fig. 1); if one cotyledon were attached to the side which becomes convex and the other to the side which becomes concave, a mechanism of unequal transport would seem more likely. Furthermore, even with the cotyledons removed, 39° of curvature was observed in hypocotyls after 72 hr (Table I).

It was also of interest to determine if lateral buds would form hooks when induced to grow. Accordingly, 4-day-old etiolated plants were decapitated just above the cotyledonary node. After 7 days in the dark, the hypocotyl hook had straightened and buds in the axils of the cotyledons were just beginning to grow out. After 7 more days, it was apparent that these new shoots had not formed hooks. When a similar experiment was performed, but with tissue above the primary leaves removed, the buds from this node grew after 7 days and also formed straight shoots. These results differ from Darwin's observations with *Vicia faba* (1) and my observations with *Pisum sativum* where lateral buds do in fact grow into shoots with hooks. The reason for the failure of bean shoots to form hooks is unclear.

Efforts were made to obtain reformation of the hook on intact plants after exposure to light. Six-day-old bean plants were placed in red light until the hypocotyl hook had either opened completely or opened only to 90°; they were then placed back into the dark for 7 more days. The hook did not reform in either set of plants. Hypocotyls of plants which had opened to 90° remained at this angle, however, and the partially opened hook eventually moved into the epicotyl, similar to the movement of completely formed hooks in etiolated plants. The ability of partially formed hooks to remain in this configuration when returned to the dark is similar to the results of Klein (5), who showed a continuous light requirement for de-etiolation of bean seedlings.

Auxins are present in bean hypocotyls (2, 11) and can not only inhibit the opening of the hook (2, 6, 7, 9, 11), but can actually intensify the curvature (2, 3). The effect of a range of concentrations of α-naphthaleneacetic acid on growth and hypocotyl hook formation in the explants was quite different than that seen for hook opening, however, as illustrated in Figure 4. The most noticeable response was the inhibition both of growth and curvature by doses of NAA higher than 0.2 mg/l; none of the concentrations of the hormone used here stimulated these two processes. The data show that inhibitions of hook formation were closely accompanied by retarded growth, but 0.2 mg/l naphthaleneacetic acid appeared to inhibit curvature slightly while having no effect on average length of the hypocotyl.

It thus appears from the results reported here that the main axis of bean plants will grow unevenly in the dark. The growth leading to this asymmetry requires the presence of the cotyledons although they probably do not cause the asymmetry *per se*. Finally, curvature and in most cases growth are inhibited by naphthaleneacetic acid concentrations above 0.2 mg/l.

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