Effects of Certain Growth Substances on the Growth and Morphogenesis of Immature Embryos of Capsella in Culture [1, 2]

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In an earlier paper (7) the development of isolated heart-shaped and older embryos of Capsella[4] (> 80 μ long) when grown in a sterile nutrient medium containing mineral salts, vitamins and 2 % sucrose was described. It was also shown that supplementing the basal medium with a mixture of growth substances like indoleacetic acid (IAA), kinetin and adenine sulfate permitted development of still smaller globular embryos (40–80 μ long) in vitro. From this evidence it seemed reasonable to assume that growth substances play a role in the development of embryos through various stages in their ontogeny. The present study describes the effects of externally applied IAA and other auxins, gibberellic acid (GA), kinetin and adenine sulfate on the growth and morphogenesis of heart-shaped and older embryos of Capsella cultured in vitro. The effects of the conditions of illumination on development are also briefly considered. The results show that embryos of different developmental stages and their respective organs show different responses to exogenous supplies of plant growth substances.

Materials and Methods

Embryos of *C. bursa-pastoris* Medic. were obtained from plants grown in the greenhouse in a mixture of equal parts of sand, perlite and leaf mold under a supplementary incandescent illumination of about 200 ft-c light during the day time. The composition of the basic culture medium and the methods of culture have been described previously (7). The growth substances tested in the experiments were added to the basal medium and autoclaved at 15 lb/in^2 for 15 minutes. Six to 8 embryos of the desired stage were routinely grown in standard 10-cm petri dishes containing 25 ml of the semi-solid medium. Cultures were kept in a culture room at 25 ± 1° and given 12 hours illumination daily by a combination of cool white fluorescent tubes and incandescent bulbs giving about 50 ft-c light on the surface of the cultures (light-grown cultures); parallel sets of cultures were kept in an incubator which was completely dark except for brief periods of opening for examination (dark-grown cultures). Transfers to fresh media were made at intervals of 4 to 5 weeks.

The morphogenetic changes occurring in the embryos were noted by periodic examination of the cultures. Growth measurements were made at the 2nd and 4th weeks from the start of the experiment, using a dissecting microscope equipped with an ocular micrometer. To get a clear picture of the effects of growth substances on the growth of the different organs in the embryos, the lengths of the cotyledons, hypocotyl and root were measured separately and their growth expressed in relative terms as the ratio final length/initial length. The root was operationally defined as the meristematic region at the proximal end and the primary root which subsequently developed from it, the cotyledons as the leafy structures at the apical notch of the embryos, and the hypocotyl as the region between the root and the cotyledons. The delimitation was arbitrary and difficult in early embryonic stages, but became easier in the later ones. Root length measurements did not include length of laterals.

All experiments were repeated 2 to 3 times using embryos of various developmental stages. In any one treatment, embryos with initial lengths close to one another within 100 μ were used. Although quantitative data on the growth of only the intermediate-stage embryos (251–450 μ long) are given, morphogenetic responses of embryos of other developmental stages are included for each treatment. Photographs of representative samples grown in the test substances are given in figures 1 to 7. Statistical methods follow Snedecor (12).

Results

Before the effects of the growth substances are considered, a brief review of the responses of the embryos when grown in the basal medium alone is essential. Our early work showed that dark-grown heart-shaped and older embryos of Capsella routinely

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[4] The different stages in the embryogenesis of Capsella, in terms of increasingly older embryos (with their range in length in microns given in parenthesis) are: early globular (20–60); late globular (61–80); early heart-shaped (81–150); late heart-shaped (151–250); intermediate stage (251–450); torpedo-shaped (451–700); walking-stick-shaped (701–1000); inverted-U-shaped (1001–1700) and mature embryos (> 1700). For details see Raghavan and Torrey (7) and Rijven (10).

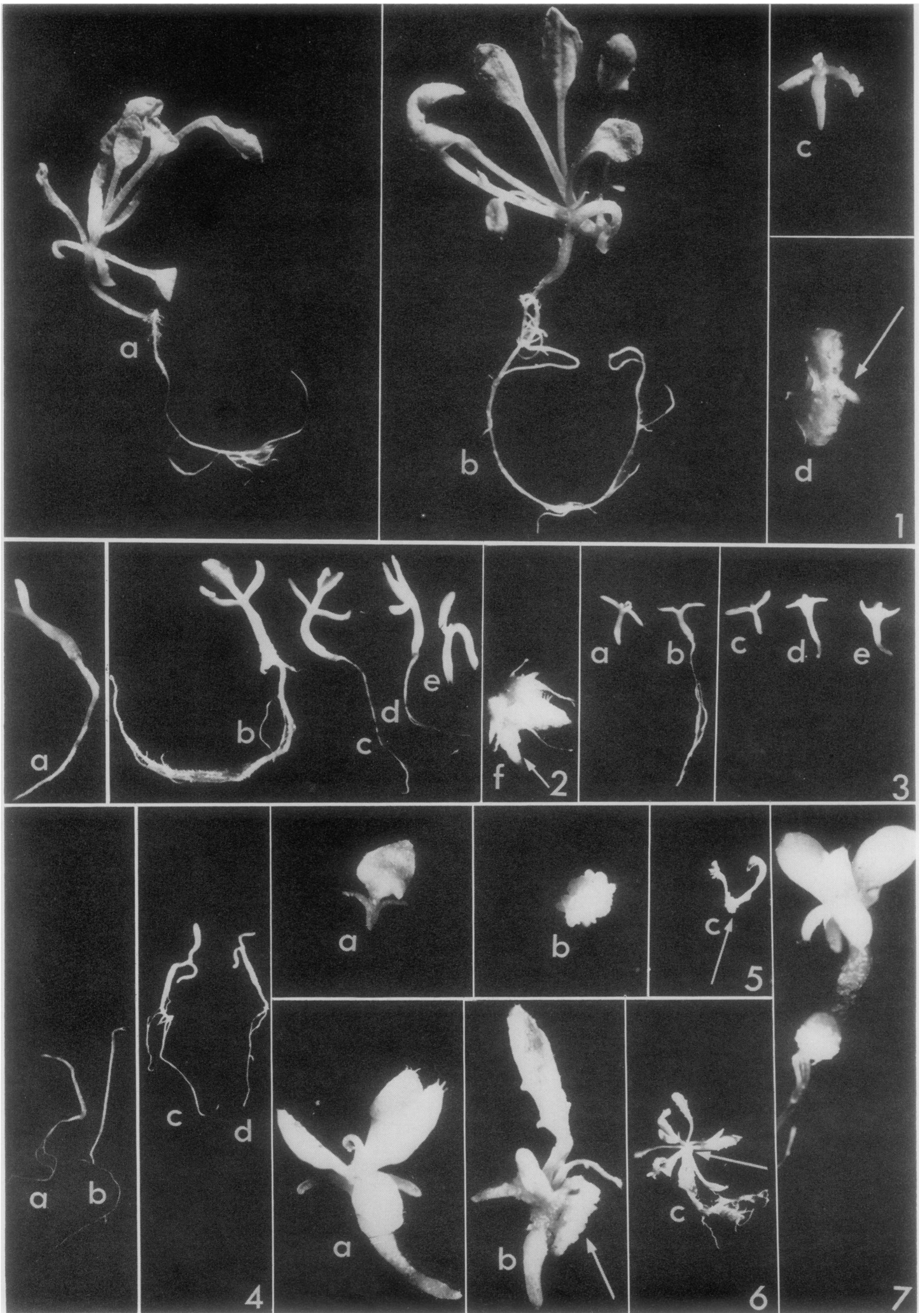
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Table I. *Growth of the Different Organs of Intermediate Stage Embryos of Capsella in Basal Medium in the Dark and in Light*

Growth is expressed as the ratio of final length/initial length ± standard error.

Organ	Dark			Light		
	Initial length, μ	Final length/initial length ± standard error		Initial length, μ	Final length/initial length ± standard error	
		2 wk	4 wk		2 wk	4 wk
Root	42.0	34.8 ± 7.9	150.4 ± 19.2	37.0	15.4 ± 2.0	25.3 ± 2.4
Hypocotyl	170.0	4.8 ± 0.1	7.1 ± 0.5	139.0	8.9 ± 0.6	12.1 ± 0.9
Cotyledons	202.0	4.0 ± 0.1	5.5 ± 0.4	152.0	7.4 ± 0.9	9.2 ± 0.6

developed a long primary root and a few lateral roots, and a shoot system composed of linear leaves. In heart-shaped through walking-stick-shaped embryos (from 81–1000 μ long) grown in light, elongation of the primary root was suppressed (fig 3a). However, in embryos > 1000 μ long (walking-stick-shaped, inverted-U-shaped, and mature embryos), root growth was not inhibited in light and such embryos formed an elaborate shoot system composed of green embryonic leaves [fig 1a; also cf fig 9–12 in (7)].

Data are presented in table I on the growth of the different organs of intermediate stage embryos during 4 weeks in culture in the basal medium. In dark-grown embryos the roots grew rapidly in 3 to 4 weeks and always surpassed in length hypocotyl and cotyledons. In light-grown embryos, growth in length of the hypocotyl and cotyledons was consistently greater than in dark-grown embryos, but the root elongated only slightly.

Effects of IAA and other Auxins. Rijven (10), and more recently Veen (18) showed that low concentrations of IAA have a slight, but significant promotive effect on the growth of torpedo-shaped embryos of Capsella. Our studies, besides confirming this observation, show that growth of embryos of all ages is promoted by IAA treatment at 0.0001 and 0.001 mg/liter. In addition, embryos of different ages grown in media containing IAA showed striking morphogenetic responses depending on the auxin concentration.

The effects of IAA on the growth in length of the different organs of dark- and light-grown intermediate stage embryos are summarized in figure 8. In the presence of low concentrations of IAA, primary root, hypocotyl and cotyledons of dark- or light-grown embryos were appreciably longer than in embryos grown in the basal medium. High concentrations of the auxin drastically inhibited growth in length of these embryonic organs.

Typical morphogenetic response of dark-grown embryos of all ages to low concentrations of IAA (0.0001 and 0.001 mg/liter) was a rapid elongation of the primary root. This was most evident between the second and fourth week (fig 2a–d): thereafter growth of the root was negligible. At these concentrations, IAA only slightly enhanced the growth of the hypocotyl and shoot in the dark. Higher concentrations of auxin inhibited growth of the primary root, hypocotyl and shoot. The cessation of hypocotyl elongation was followed by thickening of the root end which finally became a brownish mass of tissue (fig 2e). Under conditions of severe growth

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FIG. 1 to 7. Representation of embryos of Capsella cultured in the basal medium supplemented with growth substances; unless otherwise indicated, all embryos were photographed after 8 weeks in culture.

FIG. 1. Inverted-U-shaped embryos grown in light in media containing IAA; a, control; b, c, and d, respectively, in 0.0001, 0.1 and 10.0 mg/liter IAA. Arrow points to the root end of the embryo. X 4.

FIG. 2. Torpedo-shaped embryos grown in dark in media containing IAA; a, control, b, c, d, e and f, respectively, in 0.0001, 0.001, 0.01, 0.1 and 10.0 mg/liter IAA. Arrow points to the root end of the embryo. X 4.

FIG. 3. Late heart-shaped embryos grown in light in media containing IAA; a, control; b, c, d and e, respectively, in 0.001, 0.1, 1.0 and 10.0 mg/liter IAA. X 4.

FIG. 4. Effect of GA on the morphogenesis of dark- and light-grown intermediate stage embryos; a, b, embryos grown in dark in 0.1 and 1.0 mg/liter GA, respectively; c, d, embryos grown in light in 0.1 and 1.0 mg/liter GA, respectively. X 4.

FIG. 5. Effect of kinetin on the morphogenesis of light-grown early heart-shaped embryos; a, grown in medium containing 0.0001 mg/liter kinetin showing precocious expansion of the leaf; b, grown in 1.0 mg/liter kinetin showing callus formation; c, same as in b, but photographed after 10 weeks to show formation of secondary leaves from the callus. a, b X 4; c X 2.5.

FIG. 6. Effect of kinetin on the morphogenesis of light-grown walking-stick-shaped embryos. Explanation for letters as in figure 5. Arrows point to the callus from which leaves or roots arise. a, b X 4; c X 2.5.

FIG. 7. An inverted-U-shaped embryo grown in light in medium containing 20.0 mg/liter adenine sulfate showing expanded embryonic leaves. Note the suppressed primary root, and the secondary roots arising from the stump of the primary root. X 4.

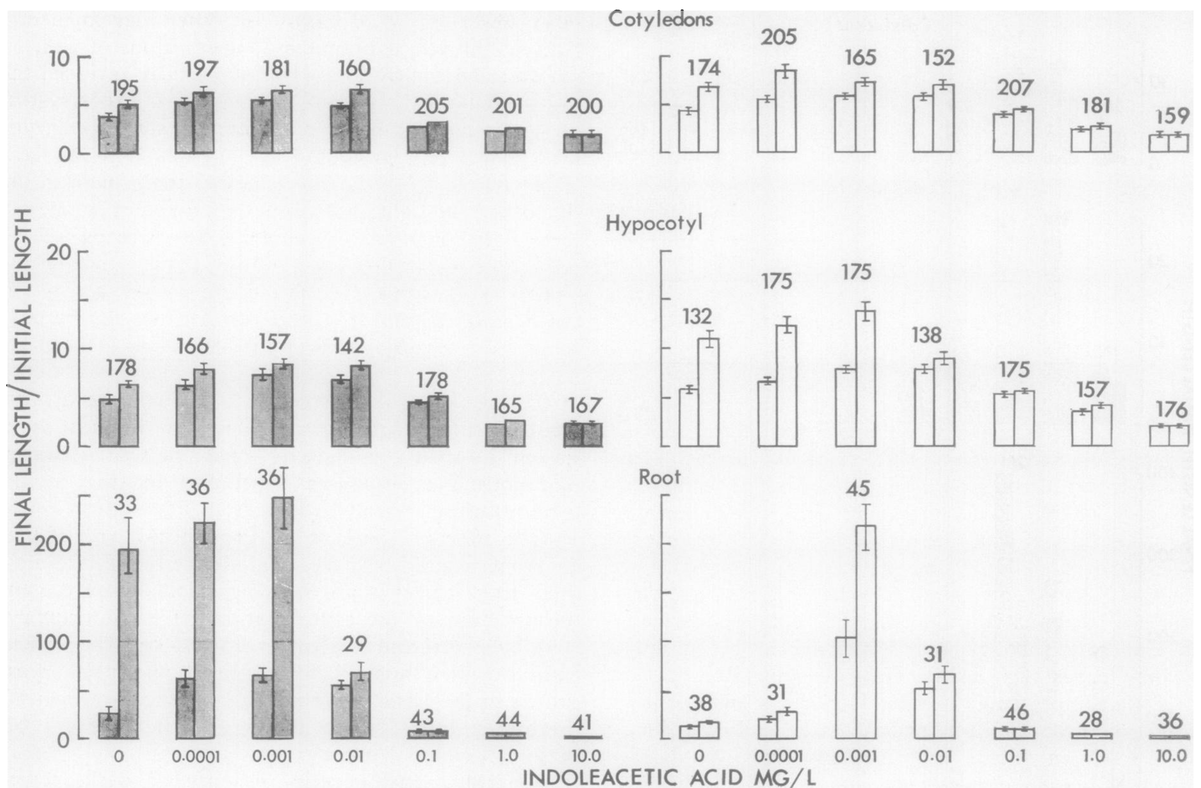

FIG. 8. The effects of increasing concentrations of IAA on the growth of the different organs of intermediate stage embryos grown in the dark (*shaded*) and in light (*unshaded*). The numbers in the graph indicate the average initial lengths (in microns) of the different organs. Vertical lines at each point represent 2 X standard error. For each concentration, values at 2 weeks (*left*) and 4 weeks (*right*) are given.

inhibition as in 10.0 mg/liter IAA, unorganized growth occurred, resulting in the formation of callus involving nearly the whole embryo (fig 2f).

In light-grown embryos, the responses to IAA were similar to those in dark-grown embryos except for two clear differences. At low auxin concentrations, elongation of the hypocotyl and cotyledons was enhanced. This effect was also apparent in somewhat older embryos; in light-grown walking-stick-shaped and older embryos (> 1000 μ long) the hypocotyl elongated dramatically in the presence of 0.0001 to 0.001 mg/liter IAA, carrying the cotyledons well above the surface of the medium and stimulating germination (fig 1a, b). The cotyledons expanded rapidly and turned green; later on, new leaves were produced. The second difference concerned the sensitivity of the root to auxin in light. While light-grown heart-shaped through walking-stick shaped embryos showed almost no root elongation in the basal medium, in the presence of IAA the roots elongated about as well as in the dark. The most effective concentrations for this response were 0.001 to 0.01 mg/liter for heart-shaped and intermediate stage embryos, and 0.0001 to 0.01 mg/liter for torpedo-shaped and older embryos (fig 3a, b).

The effects on embryo development in vitro of naphthaleneacetic acid (NAA), indoleacetonitrile (IAN) and 2,4-dichlorophenoxyacetic acid (2,4-D) were also studied. Both NAA and IAN caused little increase in growth over the controls even in the lowest concentration tested (0.0001 mg/liter) and inhibited growth of the primary root system in light- and dark-grown walking-stick-shaped and older embryos. However, paralleling the responses shown by IAA, they promoted growth of the root in light- and dark-grown torpedo-shaped and younger embryos at concentrations of 0.0001 and 0.001 mg/liter. In contrast, 2,4-D inhibited growth of the root in light- and dark-grown embryos of all ages at all concentrations. With respect to the growth of the hypocotyl and cotyledons, NAA, IAN, and 2,4-D elicited responses of the same magnitude as did IAA. At higher concentrations the effect of all auxins was one of inhibition. It consisted in thickening of the cotyledons and hypocotyl leading to callus formation.

Effects of GA. Veen (16, 18) showed that GA greatly increased the growth of torpedo-shaped embryos of Capsella and stimulated root meristem activity in embryos grown in vitro. We found that addition of GA to a medium lacking IAA promoted growth in length of embryos of all developmental stages cultured in the light or dark. Walking-stick-shaped, inverted-U-shaped and mature embryos showed optimum response at 100.0 mg/liter, torpedo-

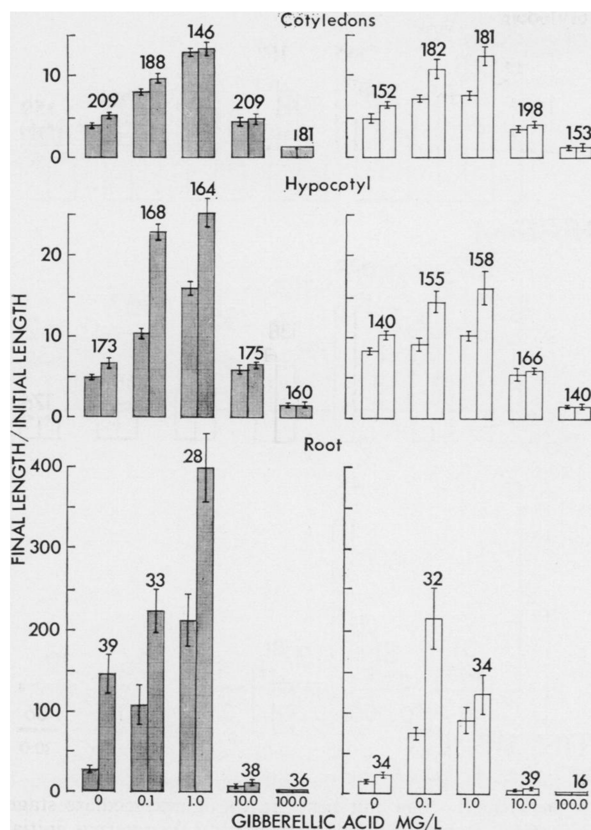

FIG. 9. The effects of increasing concentrations of GA on the growth of the different organs of intermediate stage embryos grown in the dark (*shaded*) and in light (*unshaded*). The numbers in the graph indicate the average initial lengths (in microns) of the different organs. Vertical lines at each point represent 2 X standard error. For each concentration, values at 2 weeks (*left*) and 4 weeks (*right*) are given.

shaped embryos at 10.0 mg/liter and heart-shaped and intermediate stage embryos at 0.1 and 1.0 mg/liter, respectively. Embryos 100 to 200 μ long were least sensitive to GA and responded by an initial increase in length without any morphogenetic changes.

The effects of different concentrations of GA on the elongation of the root, hypocotyl and cotyledons of intermediate stage embryos are summarized in figure 9. In dark-grown embryos, the most striking effect of GA was a stimulation of growth of the primary root at low concentrations (0.1 and 1.0 mg/liter; fig 4a, b). Elongation of the root which normally occurred after 2 to 3 weeks of culture was observed in 4 or 5 days in the presence of GA. A second effect of GA was in the elongation of the hypocotyl which raised the seedlings a few millimeters above the level of the medium. In intermediate stage embryos, elongation of the cotyledons was stimulated at low concentrations of GA for the first few weeks and thereafter the cotyledons were arrested in their development. These morphogenetic responses were also observed in embryos of other developmental stages cultured at optimum concentrations of GA.

Like IAA, low concentrations of GA reversed the light inhibition in growth of the primary root system in heart-shaped through walking-stick shaped embryos and restored the growth of the root nearly to the dark rate (fig 4c, d). GA also enhanced the growth of the hypocotyl in light, but growth of this organ lagged behind its dark-grown counterpart. Although there was slight expansion and greening of the cotyledonary leaves at the apex, GA clearly suppressed growth of embryonic leaves in light-grown embryos. The only exception to this were light-grown walking-stick-shaped and older embryos grown in media containing 100.0 mg/liter GA. Here the cotyledons rapidly expanded and became green, followed by the production of 2 to 3 pairs of leaves. Such plantlets appeared like normal seedlings. At high concentrations of GA all organs of intermediate stage embryos were markedly inhibited in their elongation whether in light or in dark.

These results show that while GA had the same effects as auxin on root development in the embryos in vitro, it differed from auxins in its effects on shoot growth. In optimum concentrations of GA, embryos of different ages exhibited a graded response with respect to the relative growth of the primary root system. Thus, walking-stick-shaped, inverted-U-shaped and mature embryos formed long primary roots with abundant laterals, while intermediate stage and torpedo-shaped embryos formed primary roots with fewer laterals. Progressively smaller embryos formed less elaborate roots. Clearly, the response to GA is markedly dependent on the developmental stage of the embryos, GA concentration and the illumination.

Effects of Kinetin and Adenine. The primary effect of kinetin on embryos grown in vitro was suppression of root growth (17, 18), but the degree of response depended upon the age of the embryos. Elongation of the primary root in dark-grown heart-shaped embryos was completely suppressed at kinetin concentrations of 0.0001 to 1.0 mg/liter; in intermediate stage embryos small root meristems 500 to 800 μ long were formed in the lowest concentration of kinetin tried (0.0001 mg/liter) while higher concentrations inhibited their growth. Figure 10 shows the predominantly inhibitory effects of kinetin on growth of root, hypocotyl and cotyledons of dark- and light-grown embryos of intermediate stage. Torpedo-shaped and older embryos grown in kinetin (0.0001–0.01 mg/liter) escaped inhibition and had long primary roots which appeared normal. Kinetin (0.1 and 1.0 mg/liter) also promoted initiation and growth of embryonic leaves in the dark-grown embryos. Leaf initiation was not, however, accompanied by internodal elongation so that short and stunted plantlets resulted. Further, the plantlets formed in media containing high concentrations of kinetin were characterized by extreme epinasty, but in contrast to the

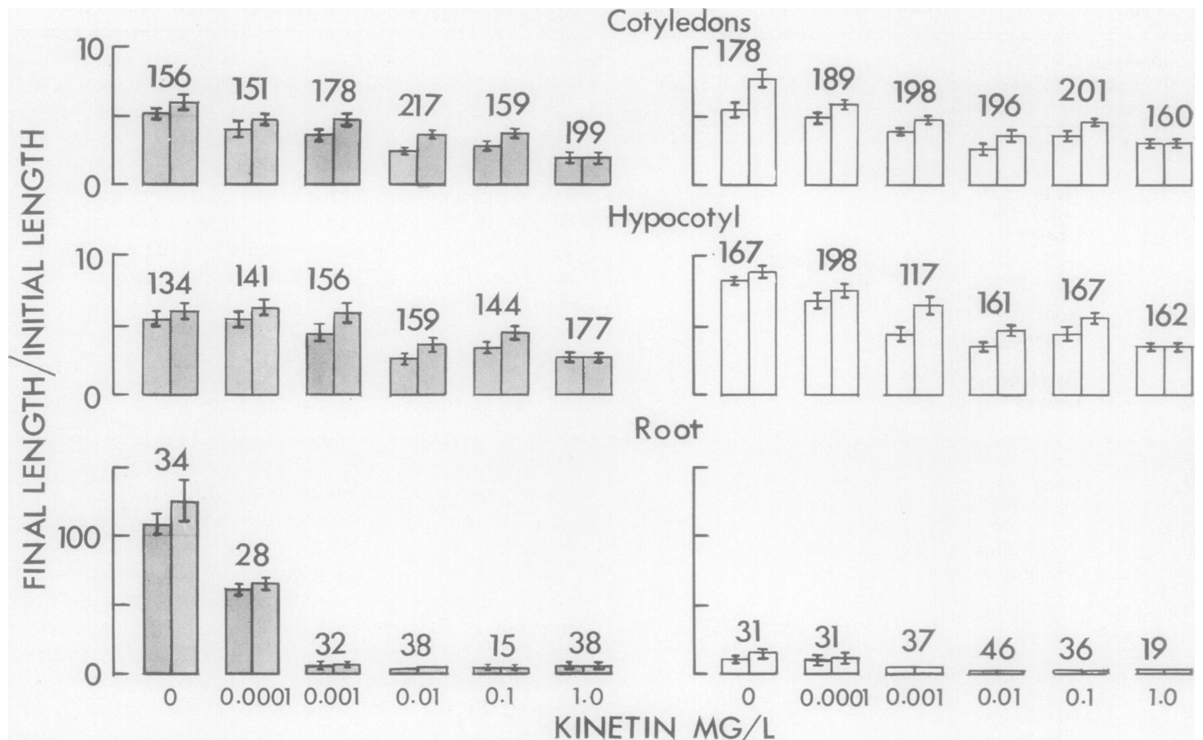

FIG. 10. The effects of increasing concentrations of kinetin on the growth of the different organs of intermediate stage embryos grown in the dark (*shaded*) and in light (*unshaded*). The numbers in the graph indicate the average initial lengths (in microns) of the different organs. Vertical lines at each point represent 2 X standard error. For each concentration, values at 2 weeks (*left*) and 4 weeks (*right*) are given.

light-grown embryos, embryos in dark rarely proliferated to form callus.

In light-grown embryos, kinetin uniformly inhibited the initiation and growth of the primary root system. Concentrations of 0.0001 and 0.001 mg/liter kinetin promoted precocious leaf initiation. Usually one large leaf appeared in early and late heart-shaped embryos, but in older embryos a more sustained effect was noted resulting in 2 or more expanded leaves (fig 5a, 6a). At concentrations of 0.1 and 1.0 mg/liter kinetin was particularly striking in its stimulatory effect on callus formation in embryos of all ages (fig 5b, 6b). A thickening of the cotyledons and hypocotyl appeared after 7 to 10 days of culture and within 4 to 5 weeks unorganized tumor-like masses had developed by the transformation of nearly the whole of the embryo into a callus, comprised of large and irregular cells and scattered nodules of tracheids. During further periods of culture, several pairs of leaves and secondary roots appeared on the callus giving the appearance of small plantlets (fig 6c, 5c). These responses are not apparent in the elongation measurements given in figure 10.

Like kinetin, adenine (adenine sulfate) inhibited growth in length and formation of the primary root system in both light- and dark-grown embryos at all concentrations tested (0.1–40.0 mg/liter). In embryos $> 700\ \mu$ long a secondary effect of adenine sulfate at concentrations above 10.0 mg/liter was the initiation and growth of several secondary roots from the stump of the suppressed primary root. In the presence of adenine sulfate, the first few pairs of leaves in light-grown embryos were characteristically deep green, nearly sessile, flat and oval with an obtuse apex (fig 7). In dark-grown embryos, there was no appreciable expansion of the leaves although several leaf primordia overlapping one another were visible at the terminal bud. Adenine sulfate was ineffective in inducing growth of the leaves in torpedo-shaped and younger embryos. The leaves appeared without any accompanying internodal elongation resulting in short and stunted plantlets.

Discussion

The effect of a combination of IAA, kinetin and adenine sulfate in initiating growth and differentiation in isolated globular embryos of Capsella in vitro was shown earlier (7). The present report shows that organ initiation and development in heart-shaped and older embryos can be controlled by supplementing the medium with substances like auxins, gibberellin, kinetin or adenine. A summary of the results of these experiments is given in table II; however the changing response of embryos of different developmental stages to the concentration of any growth substance is not fully evident from such a table.

The effect of applied auxin in the initiation and

Table II. *Effect of Growth Substances on the Growth and Differentiation of Dark- and Light-Grown Embryos*

Supplements to the basal medium	Organ	Dark		Light	
None (cf Table I)	Root	++*		< 1000 μ long 0 > 1000 μ long ++	
	Hypocotyl	+		++	
	Cotyledons	+		++	
	Leaves	+		++	
		Low (0.0001–0.01 mg/liter)	*High (0.1–10.0 mg/liter)*	*Low*	*High*
IAA	Root	+++	—	++	—
	Hypocotyl	+	—	< 1000 μ long +	—
			Callus	> 1000 μ long ++	— Callus
	Cotyledons	+	—	++	—
	Leaves	++	—	++	—
		Low (0.1–1.0 mg/liter)	*High (10.0–100.0 mg/liter)*	*Low*	*High*
GA	Root	++++	—	+++	—
	Hypocotyl	+++	< 1000 μ long + or 0 > 1000 μ long +++	+	< 1000 μ long — or 0 > 1000 μ long ++
	Cotyledons	++	0	++	— or 0
	Leaves	—	—	—	< 1000 μ long — > 1000 μ long ++
		Low (0.0001–0.001 mg/liter)	*High (0.01–1.0 mg/liter)*	*Low*	*High*
Kinetin	Root	< Torpedo-shaped — > Torpedo-shaped ++	— —	— —	— —
	Hypocotyl	—	—	—	— Callus
	Cotyledons	—	—	—	—
	Leaves	0	++	++ (Precocious expansion)	++ (Secondary leaves)
		Low (0.1–10.0 mg/liter)	*High (20.0–40.0 mg/liter)*	*Low*	*High*
Adenine sulfate	Root	—	—	—	—
	Hypocotyl	—	—	—	—
	Cotyledons	—	—	—	—
	Leaves	—	< Torpedo-shaped — > Torpedo-shaped ++ (Primordia)	—	< Torpedo-shaped — > Torpedo-shaped ++

* Explanation of signs: +, stimulation; 0, no effect; —, inhibition.

growth of the root system in embryos grown in vitro has been described by several authors (1, 2, 5, 8, 11). The stimulation of root growth in low concentrations of IAA suggests that initiation and continued growth of the root system in embryos may involve the positive action of auxin. The complete inhibition of root formation in embryos < 1000 μ long when grown in the basal medium in light and the reversal of this inhibition by IAA indicate that light causes inactivation of an auxin-like substance necessary for the formation of the root system. The observation that the light-grown heart-shaped and intermediate stage embryos required a higher concentration (0.001 mg/liter) of IAA for development of the root system than dark-grown embryos of comparable age, is also consistent with the idea of a light-dependent destruction of auxin in the embryos.

The results also show a general promotion in growth of the hypocotyl and shoot system by low concentrations of IAA. Of interest is the fact that auxin is more effective in light- than in dark-grown embryos. If the growth of the shoot is assumed to require a lower range of auxin in the medium than initiation and growth of the primary root, a light-activated destruction of auxin will also explain the better growth of the shoot system in light-grown embryos, while, conceivably in dark-grown embryos this growth may be inhibited by supraoptimal concentrations of auxin.

More effective than auxin in promoting growth of the root system in embryos in vitro was GA, a substance known for its pronounced effects on stem and leaf expansion in many species of vascular plants (13). Most workers seem to agree that GA exerts a detrimental effect on the growth of roots, although some reports have pointed to the contrary (2, 19). While promoting growth of the root system in Capsella embryos, GA inhibited formation of embryonic leaves and the characteristic GA-induced leaf and shoot responses seen in other plants were not observed. Similar effects of GA in organ initiation have been reported by Brown and Gifford (2) in pine embryos.

A number of morphogenetic studies indicate that kinetin promotes shoot and bud initiation, but inhibits the growth of roots in higher plants (6). Our results show that kinetin produces these same responses on the shoot and root systems of the cultured embryos. With respect to the development of the shoot system in the embryos, kinetin was more active in light than in dark; on the other hand, in dark-grown embryos, growth of the root was only weakly inhibited in the presence of low concentrations of kinetin. Similar effects of kinetin in the presence or absence of illumination have been reported (4, 14). The structural feature of kinetin responsible for callus formation does not appear to be due solely to the purine nucleus, since adenine which is the purine component of kinetin was ineffective in inducing callus growth in the embryos.

Embryos of different ages tend to fall in respect to their responses, into 3 broad categories which will be tentatively designated as proembryos (embryos $< 80\ \mu$ long, comprising early and late globular stages), immature embryos (81–700 μ long, comprising early heart-shaped through torpedo-shaped embryos) and differentiated embryos ($> 701\ \mu$ long, comprising walking-stick-shaped through mature embryos). Results of experiments with auxins and other growth substances, tested individually indicate that it is unlikely that there is a single essential compound necessary to induce growth in proembryos. Rather, continued growth and development of proembryos was possible due to interaction of a balanced mixture of IAA, kinetin and adenine sulfate. Still younger embryos, down to the minimum size of about 20 μ long, have not yet responded to the manipulation of hormone mixture, but it seems possible that the balance of the same hormones, perhaps supplemented by others, is also critical here. This picture of the complex hormonal and nutritional requirements of younger embryos fits in with the evidence from other studies on the culture of plant embryos (3, 9, 10, 15).

Once differentiation has started, the immature and differentiated embryos are capable of developing into normal plants even in a medium lacking the growth substances, evidently drawing on endogenous hormone supplies. In both immature and differentiated embryos, growth of root, leaves and shoot was inhibited or promoted by IAA, kinetin or adenine sulfate. Thus, marshalling of the cells into different organs in these embryos may be determined by the same set of factors that control differentiation in the proembryos. Based on the age changes in sensitivity to supplied growth substances one may infer a progressive increase in endogenous auxin levels from early globular to later stages of differentiated embryos and a concurrent decrease in endogenous GA levels. Veen (18) has suggested a similar patterned distribution of kinin in inducing morphogenetic changes in embryos of Capsella, although he found no evidence for IAA or GA in such a role. It thus seems clear that early embryogenesis involves a complicated and rapid sequence of changing hormonal interactions. It will, however, be necessary to examine hormonal levels during embryo development more directly in order to determine how these hormones act in organ initiation during development.

Summary

The effects of certain growth substances on the growth and morphogenesis of heart-shaped and older embryos of *Capsella bursa-pastoris* Medic. in vitro were studied. Indoleacetic acid at 0.0001 and 0.001 mg/liter promoted growth of the primary root, hypocotyl and cotyledons and induced initiation of embryonic leaves in both light- and dark-grown embryos of all ages. Higher concentrations of indoleacetic acid were proportionately less effective and 1.0 and 10.0 mg/liter caused callus formation. The responses of embryos to naphthaleneacetic acid, indoleacetonitrile, and 2,4-dichloropheneoxyacetic acid were similar. Gibberellic acid was more effective than the auxins in promoting elongation of the root and hypocotyl in light- and dark-grown embryos. Gibberellic acid inhibited shoot initiation at the apices of embryos of all ages except in light-grown walking-stick-shaped and older embryos. Kinetin (0.0001 to 1.0 mg/liter) was inhibitory to growth of the primary roots and at 0.0001 and 0.001 mg/liter promoted precocious expansion of the embryonic leaves. In 0.1 and 1.0 mg liter kinetin, light-grown embryos formed callus which subsequently differentiated leaves and secondary roots. Adenine sulfate also inhibited root growth in embryos of all ages, and induced precocious leaf expansion in walking-stick-shaped and older embryos. The possible role of a changing balance of plant hormones during normal embryogenesis is discussed.

Literature Cited

1. Ball, E. 1959. Growth of the embryo of *Ginkgo biloba* under experimental conditions. III. Growth rates of root and shoot upon media absorbed through the cotyledons. Am. J. Botany 46: 130–39.
2. Brown, C. L. and E. M. Gifford, Jr. 1958. The relation of the cotyledons to root development of pine embryos grown in vitro. Plant Physiol. 33: 57–64.
3. DeMaggio, A. E. and R. H. Wetmore. 1961. Morphogenetic studies on the fern *Todea barbara*. III. Experimental embryology. Am. J. Botany 48: 551–65.
4. Fries, N. 1960. The effect of adenine and kinetin on the growth and differentiation of Lupinus. Physiol. Plantarum 13: 468–81.
5. Jayasekera, R. D. E. and P. R. Bell. 1959. The effect of various experimental treatments on the development of the embryo of the fern *Thelypteris palustris*. Planta 54: 1–14.
6. Miller, C. O. 1961. Kinetin and related compounds in plant growth. Ann. Rev. Plant Physiol. 12: 395–408.
7. Raghavan, V. and J. G. Torrey. 1963. Growth and morphogenesis of globular and older embryos of Capsella in culture. Am. J. Botany 50: 540–51.

8. RIETSEMA, J., S. SATINA, AND A. F. BLAKESLEE. 1953. The effect of indole-3-acetic acid on Datura embryos. Proc. Natl. Acad. Sci. 39: 924–33.
9. RIETSEMA, J., S. SATINA, AND A. F. BLAKESLEE. 1953. The effect of sucrose on the growth of *Datura stramonium* embryos in vitro. Am. J. Botany 40: 638–46.
10. RIJVEN, A. H. G. C. 1952. In vitro studies on the embryo of *Capsella bursa-pastoris*. Acta Botan. Neerl. 1: 157–200.
11. SACHER, J. A. 1956. Observations on pine embryos grown in vitro. Botan. Gaz. 117: 206–14.
12. SNEDECOR, G. W. 1956. Statistical Methods. The Iowa State College Press, Ames.
13. STOWE, B. B. AND T. YAMAKI. 1957. The history and physiological action of the gibberellins. Ann. Rev. Plant Physiol. 8: 181–216.
14. TORREY, J. G. 1958. Endogenous bud and root formation by isolated roots of Convolvulus grown in vitro. Plant Physiol. 33: 258–63.
15. VAN OVERBEEK, J., M. E. CONKLIN, AND A. F. BLAKESLEE. 1942. Cultivation in vitro of small Datura embryos. Am. J. Botany 29: 474–77.
16. VEEN, H. 1961. The effect of gibberellic acid on the embryo growth of *Capsella bursa-pastoris*. Proc. Koninkl. Ned. Akad. Wetenschap. (C) 64: 79–85.
17. VEEN, H. 1962. Preliminary report on effects of kinetin on embryonic growth in vitro of Capsella embryos. Acta Botan. Neerl. 11: 228–29.
18. VEEN, H. 1963. The effect of various growth-regulators on embryos of *Capsella bursa-pastoris* growing in vitro. Acta Botan. Neerl. 12: 129–71.
19. WHALEY, W. G. AND J. KEPHART. 1957. Effect of gibberellic acid on growth of maize roots. Science 125: 234.

Oxidation of Externally Added NADH by Isolated Corn Root Mitochondria [1, 2]

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Introduction

The permeability of mitochondrial membranes and the accessibility of external substrates to intramitochondrial enzyme systems have been suggested to be mechanisms for the control of cellular metabolism (16, 17, 19). Lehninger (9) showed that the oxidation of extra-mitochondrial NADH could be enhanced by osmotically damaging mitochondrial structure. Linnane and Ziegler (11; see also 5, 8, 10) have shown that intact mitochondria isolated from beef hearts will not oxidize externally added NADH, while mitochondrial fragments will. Other workers (3, 14, 18) have reported that mitochondria, which they consider to be well preserved, are able to oxidize external NADH.

Bonner and Voss (2) have stated that mitochondria isolated from a number of plant tissues are able to oxidize external NADH while structurally intact. They suggest that this ability is a phenomenon common to plant tissues, and that it represents one of the few marked differences between plant and animal mitochondria.

Contradictory reports of the activities of intact mitochondria can often be traced to a lack of agreement on the criteria which most truly indicate intactness. Schneider (15) has pointed out the directness and sensitivity of electron microscope observations in the determination of the identity and state of cellular organelles. Studies of NADH oxidation in isolated mitochondria, particularly plant mitochondria, have rarely included electron microscope observations among the evidences for structural intactness or disruption. This report presents some electron micrographs of mitochondria isolated from a plant tissue and discusses the relationship between oxidative activity and structure.

Materials and Methods

Preparation of Mitochondria. Mitochondria were isolated from 5 mm apical segments of *Zea mays* L. seedlings freshly grown in the laboratory from the single cross hybrid line, University of Texas 854x857. Dry seeds were washed with a mild detergent, rinsed thoroughly in distilled water, rolled in filter paper saturated with distilled water, germinated, and grown for 5 days in a darkened cabinet at 25°. The roots were harvested by removing the seedlings individually and excising the root apices with a razor blade. The root segments were gathered as they were cut in a beaker stored in an ice bucket. All further isolation operations were carried out at 0 to 4°.

The segments were washed several times in cold 0.5 M sucrose, drained, and minced with a razor blade on a glass plate. The material was divided into batches, each of which contained about 1 g fresh weight of root tips, and each batch was homogenized for one minute with 10 ml of 0.5 M sucrose solution

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