THE GROWTH CAPACITY OF THE SUNFLOWER HYPOCOTYL

R. S. DE ROPP[1]

(WITH TWO FIGURES)

Received February 26, 1951

Since the early studies of SACHS (2) on the elongation of roots and stems, it has been realized that the capacity for growth of these plant organs is not equal along the whole of their lengths but varies from one region to another. The present study was designed to give information as to the growth capacities of excised fragments taken from the different regions of sunflower hypocotyl. The growth of these fragments was studied under three sets of conditions: (1) on sucrose mineral agar, (2) on sucrose mineral agar to which indole-3-acetic acid (IAA) had been added, (3) on sucrose mineral agar after inoculation of the fragment with crown-gall bacteria. Five aspects of growth were measured: increase in length, breadth, fresh and dry weight, and production of adventitious roots.

Sunflower seeds var. Russian Giant were sterilized by being immersed for two minutes in a 1% solution of sodium hypochlorite. They were then washed in three changes of sterile distilled water and soaked in water for two hours. The embryos were then removed from their seed coats, transferred aseptically to tubes (25 mm. × 200 mm.) each containing 10 ml. nutrient agar (2% sucrose + White's mineral solution + 1% agar) and cultured at 25° C in continuous light from four 40 watt fluorescent lamps. When the hypocotyls attained a length of 100 mm. the embryos were drawn out of their tubes and the hypocotyls severed just above the root and immediately below the cotyledons. The hypocotyls were cut into segments five mm. long which were placed radicle end uppermost in agglutination tubes (10 mm. × 75 mm.) containing three ml. of nutrient agar. They were incubated for 10 days in darkness at 25° C, then removed, photographed, and weighed. The photographs were made by projecting the image of the fragment through an enlarger onto printing paper. The magnification used was 1.7 diameters in most instances. The length and breadth of the fragment and the size of the tumors were estimated from these shadowgraphs. *Agrobacterium tumefaciens*, strain B.P., was used in these experiments, suspensions being prepared from 24 hour slopes. Indoleacetic acid was incorporated into the nutrient agar for the hypocotyl fragments after autoclaving.

Growth of hypocotyl fragments on sucrose mineral agar

Ten sunflower hypocotyls, each about 100 mm. long, were divided into 20 segments five mm. long; and every alternate fragment beginning with

[1] Present address: Lederle Laboratories Division, Amer. Cyanamid Co., Pearl River, New York.

the one nearest the cotyledons was placed in a tube of sucrose mineral agar. The distances of the foliar ends of these fragments from the points of attachment to the cotyledons were thus: 0, 10, 20, 30, 40, 50, 60, 70, 80, and 90 mm. After ten days of incubation in continuous darkness at 25° C the fragments were removed, photographed; and the fresh and dry weights were determined. The mean length, breadth, fresh and dry weight of the fragment, the number of fragments with adventitious roots, and mean number of roots per fragment were measured (table I, fig. 1).

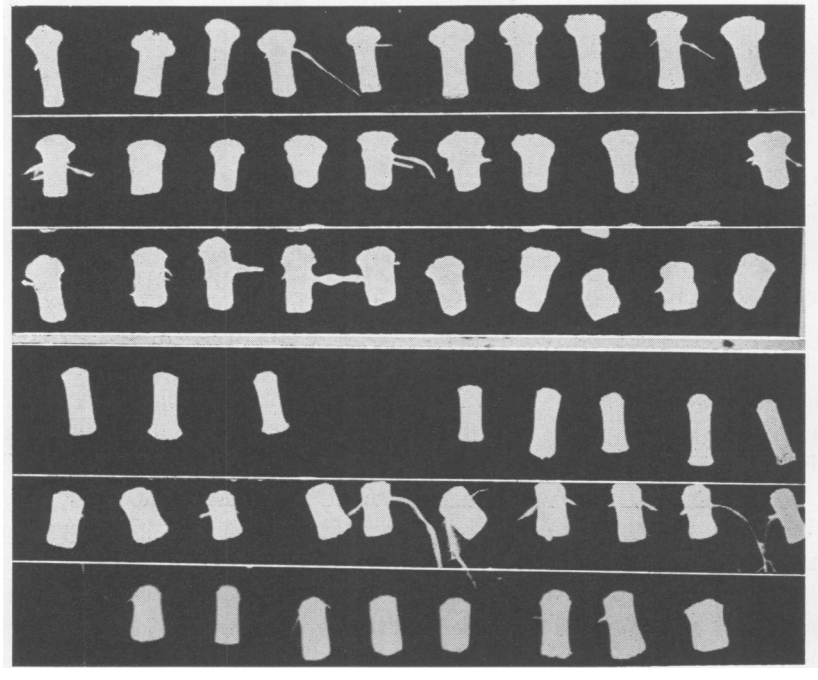

FIG. 1. Upper three rows: fragments of sunflower hypocotyl excised 0 mm. (top row), 40 mm. (middle row) and 90 mm. (bottom row) from point of attachment of cotyledons. Inoculated with crown-gall bacteria, cultured 10 days on nutrient agar. Lower three rows: uninoculated fragments from the same three regions.

Growth in length was greatest in fragments taken from the apex of the hypocotyl, though the fragments in the group nearest the base also elongated significantly more than did those from the center. Increase in width was least in the apical fragments but was about equal in all fragments from 20 mm. down. Production of adventitious roots did not occur at all on the fragments of the apical group but increased in frequency through the 10, 20, and 30 mm. groups, attaining a maximum value in the 40 mm. and lower groups. Numbers of roots per fragment did not differ significantly amongst groups below 10 mm. Both mean fresh and dry weights were significantly higher in groups below the 10 mm. level.

TABLE I

GROWTH ON SUCROSE MINERAL AGAR OF FIVE MM. FRAGMENTS FROM DIFFERENT REGIONS OF THE SUNFLOWER HYPOCOTYL (MEANS OF 10 ESTIMATES)

Distance from cotyledons	Length	Breadth	Weight		Fragments with roots	Roots per fragment
			Fresh	Dry		
mm.	*mm.*	*mm.*	*mg.*	*mg.*		
0	7.0	2.3	29.5	2.3	0	0
10	6.2	2.5	32.1	2.3	3	2
20	5.8	2.8	34.1	2.5	3	3
30	5.0	2.7	36.0	2.8	5	3
40	5.5	2.8	39.3	2.8	7	2
50	5.8	2.8	37.8	3.0	8	2
60	5.6	2.8	37.6	3.0	7	2
70	5.2	3.0	41.0	3.1	8	2
80	5.8	3.0	44.0	3.1	8	2
90	6.4	3.0	31.3	3.0	8	2

Growth of fragments inoculated with crown-gall bacteria

This experiment was similar to the previous one; but the hypocotyl fragments, after being placed on nutrient agar, were inoculated on the freshly cut upper surface with about 1×10^7 viable organisms of *Agrobacterium tumefaciens* strain B.P. These fragments were also weighed and photographed at the end of 10 days incubation in continuous darkness (table II, fig. 1).

Many changes in the growth pattern of these fragments were induced by inoculation with crown-gall bacteria. The increase in length reached a maximum in the two apical groups and was significantly greater than was the increase in the corresponding uninoculated series. The maximum breadth was considerably increased owing to tumor formation on the inoculated end of the fragment. The breadth of the tumors was about the same

TABLE II

GROWTH OF FRAGMENTS OF SUNFLOWER HYPOCOTYL INOCULATED WITH CROWN-GALL BACTERIA (MEANS OF 10 ESTIMATES)

Distance from apex	Length	Breadth	Weight		Fragments with roots	Roots per fragment
			Fresh	Dry		
mm.	*mm.*	*mm.*	*mg.*	*mg.*		
0	7.7	4.3	43.7	2.9	7	2
10	6.9	4.2	41.4	2.9	7	3
20	6.4	4.0	38.2	2.3	7	4
30	6.0	4.0	38.6	2.5	6	4
40	6.0	4.2	40.0	2.8	5	5
50	6.1	4.0	41.5	3.1	3	6
60	5.7	4.1	41.1	3.2	6	4
70	5.9	3.6	36.3	2.9	4	4
80	6.1	4.0	41.8	2.9	4	4
90	6.2	3.7	41.8	2.5	7	3

in all 10 groups. The frequency of occurrence of adventitious roots was greatest in the apical segments, and the mean number of adventitious roots was greatest in the central segments. Both frequency and mean number of roots per fragment were significantly higher in the fragments of the inoculated group as compared with those of the uninoculated group. The fresh and dry weights of the apical fragments in the inoculated group were significantly higher than those of the corresponding uninoculated group.

Growth of fragments in the presence of IAA

To determine the effect of the position of the fragment on its reaction to IAA, 40 sunflower hypocotyls were divided into fragments 5 mm. long. Only the fragments at 0, 30, 60, and 95 mm. from the apex were used. Forty fragments from each level were divided into four groups of 10 fragments each. Fragments in group 1 were placed on agar containing 1 mg./l. IAA, those in group 2 on 0.1 mg./l. IAA, those in group 3 on 0.01 mg./l. IAA, those in group 4 on agar devoid of IAA. The experiment thus contained fragments from four regions of the hypocotyl on four concentrations of IAA, each treatment being replicated 10 times, involving 160 fragments in all.

After 10 days of incubation in continuous darkness at 25° C the fragments were removed, photographed and weighed (table III, fig. 2). In the presence of IAA, the elongation of fragments in the apical group was increased as compared with that of the controls. This effect of IAA on elon-

TABLE III

GROWTH OF FRAGMENTS FROM FOUR REGIONS OF THE SUNFLOWER HYPOCOTYL ON NUTRIENT AGAR CONTAINING IAA

IAA conc.	Distance from apex	Length	Breadth	Weight		Fragments with roots	Roots per fragment
				Fresh	Dry		
mg./l.	*mm.*	*mm.*	*mm.*	*mg.*	*mg.*		
1.0	0	9.7	6.2	100.0	7.7	5	2
	30	6.8	5.2	84.0	6.2	8	4
	60	6.4	6.0	87.0	7.5	5	2
	95	6.4	5.5	107.5	8.2	8	2
0.1	0	9.1	6.0	95.5	6.1	9	3
	30	6.9	5.2	88.5	5.5	8	11
	60	6.2	5.4	93.7	6.2	8	10
	95	6.6	5.7	98.2	7.0	8	6
0.01	0	9.6	4.2	52.2	4.0	3	2
	30	5.8	5.1	60.0	4.4	9	6
	60	5.8	5.0	61.0	4.4	7	6
	95	6.4	5.2	75.7	5.3	8	2
0	0	7.0	2.9	28.9	2.7	0	0
	30	5.7	3.5	41.3	3.2	5	2
	60	5.2	3.2	40.0	3.2	4	2
	95	6.4	3.1	40.0	3.0	4	1

gation was less marked on fragments excised from the lower regions of the hypocotyl. The breadth of the fragments increased greatly in the presence of IAA, this effect being greatest on the 0.1 and 1.0 mg./l. concentrations. The increase in breadth was due in part to the formation of the

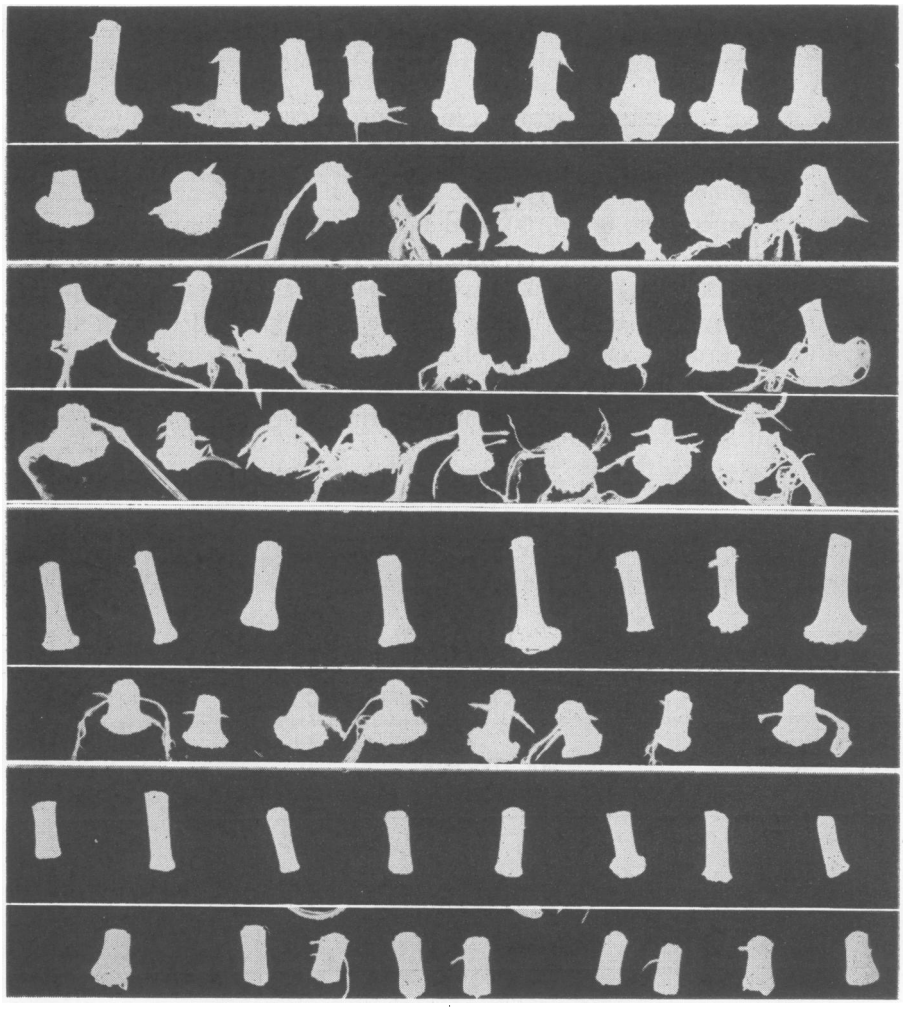

FIG. 2. Fragments of sunflower hypocotyl cultured on nutrient agar containing 1.0 mg./l. IAA (rows 1 and 2), 0.1 mg./l. IAA (rows 3 and 4), 0.01 mg./l. IAA (rows 5 and 6), 0 mg./l. IAA (rows 7 and 8). Fragments in upper of each pair of rows excised 0 mm. from point of attachment of cotyledons. Fragments in lower row excised 95 mm. from this point.

characteristic hyperhydric tissue which has already been described for sunflower stem fragments cultured in the presence of these concentrations of IAA (1). Fragments from levels 60 mm. and 95 mm. sometimes became almost spherical in outline as a result of this growth (fig. 2). In the group of fragments from the apex of the hypocotyl, the swelling was confined to

the surface of the fragment in contact with the agar. Maximum adventitious root production occurred on those fragments which were in contact with 0.1 mg./l. IAA. The mean number of such roots was greatest in fragments on levels 30 and 60 mm. and least on levels 0 and 95 mm. Production of adventitious roots occurred mainly at two regions on the fragments in contact with IAA, one group arising near the radicle end of the fragment, the other from the end in contact with the agar (fig. 2).

The IAA brought about a considerable increase in both fresh and dry weights of these fragments, the greatest increase occurring in the presence of 1 and 0.1 mg./l. of this substance. IAA also eliminated the difference in weight between fragments from level 0 and those from lower levels which was considerable in those fragments cultured in the absence of IAA.

The aim of these studies was to determine the relationship between the capacity for growth of isolated fragments of sunflower hypocotyl and the region of this organ from which they were excised. Capacity for elongation was localized mainly in the 10 mm. of the hypocotyl immediately below the point of attachment of the cotyledons. This capacity was enhanced both by inoculating the fragment with crown-gall bacteria and by culturing the fragment in the presence of 0.01 mg./l. IAA. Fragments from the upper region of the hypocotyl, when cultured on sucrose and mineral agar, had a smaller capacity for growth in breadth and in fresh and dry weight than had those taken from the lower regions. This difference was eliminated when 1.0 or 0.1 mg./l. of IAA was present in the medium. Inoculation with crown-gall bacteria also largely eliminated these differences. Capacity for production of adventitious roots was lowest in fragments taken from the upper 10 mm. of the hypocotyl. Root production by these fragments was enhanced by the presence of 0.1 mg./l. IAA in the medium but still remained less than that of fragments from the central region grown on a similar medium. Inoculation with crown-gall bacteria also enhanced root production. Fragments from the extreme base of the hypocotyl developed fewer roots than did fragments from the central region. Capacity for tumor production in response to inoculations with crown-gall bacteria was about equal in all fragments.

This work was done in part on a grant in aid recommended by the Committee on Growth of the National Cancer Society.

NEW YORK BOTANICAL GARDEN
NEW YORK, NEW YORK

LITERATURE CITED

1. DE ROPP, R. S. The response of normal plant tissue and of crown-gall tumor tissues to synthetic growth hormones. Amer. Jour. Bot. **34**: 53–62. 1947.
2. SACHS, J. Textbook of Botany. Oxford Univ. Press, New York. 2nd Ed. 1882.