Plant Growth-stimulating Hormones in Immature Bean Seeds

John W. Mitchell, Dorothy P. Skaggs, and W. Powell Anderson

Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, USDA, Beltsville, Maryland

Immature seeds of plants such as apple, corn, rye, and wheat contain extractable phytohormones (*1–6*). Presence of these substances in seed extracts has been detected through their accelerating effect on the elongation of cells in oat coleoptiles and through their stimulating effect on the growth of tomato and pepper fruits. In studying these seed hormones, investigators have most often applied them to plants of different species from those from which they were taken.

In the present investigation, which was carried out under controlled greenhouse conditions, ether-soluble hormones obtained from the immature seeds of Black Valentine bean plants were applied to seedling plants of the same variety. Vegetative growth was accelerated in this way, and the plants were made to develop flowers 3–5 days earlier than untreated ones. The magnitude of the response reflected in vegetative growth varied, depending on the stage of development of the seeds from which the extracts were made.

Under the conditions of the experiment, the amount of ether-soluble hormones increased rapidly in the seeds after the fourth day following pollination, reaching a maximum on about the seventh or eighth day, and then decreased rapidly to an immeasurable amount on about the fifteenth day. The most potent extracts were obtained from seeds 3–4 mm long taken from pods that varied from 12 to 13 cm in length. Maximum rate of pod growth (increase in pod length) occurred during the first 8–10 days following pollination. Growth of the seeds (fresh weight) was most rapid subsequent to this period. The peak of extractable hormones was more closely associated with pod growth than with seed growth, but this does not mean that the hormones necessarily influenced growth of the pods (Fig. 1). The lack of extractable hormones in the case of relatively mature seeds was not due to the thickened seed coats, since these seeds, when chopped into small pieces and soaked in ether, failed to yield a measurable amount of hormones.

The extracts were prepared by soaking a freshly collected sample of whole seeds in moist ether at room temperature for 18–24 hr. After the ether was separated and evaporated, lanolin was mixed with the residue to afford the desired concentration of extract in the fatty carrier. In testing this method, the residue obtained from evaporating ether alone was mixed with lanolin and the mixture was applied to test plants, with no apparent effect.

In measuring the potency (cell-elongating activity) of an extract, lanolin paste containing a known amount of the extract was applied quantitatively and unilaterally to the first internode of each seedling. The concentration of extract in the lanolin was varied during repeated trials, and the weight of extract required to cause a slight curvature of the stems was determined. Total activity of an extract was calculated by multiplying this value by the weight of extract obtained per seed. This method was used to measure the amount of extractable hormones in seeds at different stages of development (Fig. 1).

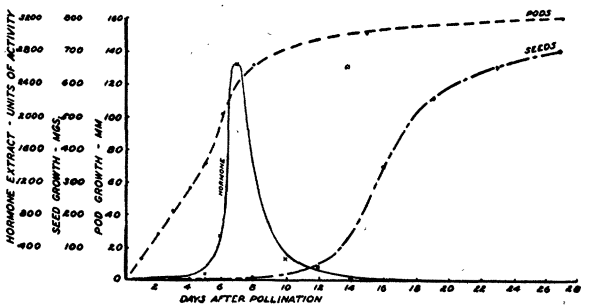

FIG. 1. Growth of bean pods and seeds in terms of length and fresh weight, respectively, compared with amount of extractable cell-elongating hormones obtained from the seeds at intervals following pollination.

In some experiments, approximately 125 μg of the crude extract was applied in lanolin as a band around the first internodes of 10 seedlings. Ten comparable untreated plants were used for comparison. The extract stimulated elongation of the treated internodes and of all internodes above the treated ones, thus causing the stems to elongate much more rapidly than did those of the untreated plants (Table 1 and Fig. 2). The time of flowering was advanced 3–5 days, and the expansion of trifoliate leaves proceeded at a much more rapid rate on the treated than on the untreated plants. The extract affected stem growth much less when applied to relatively mature bean plants having several expanded trifoliate leaves than when applied to seedlings.

The extract stimulated growth of the seedlings when applied to the young succulent portions of their

TABLE 1

AVERAGE LENGTH (MM) OF INTERNODES OF BEAN PLANTS TREATED WITH 125 μG CRUDE SEED EXTRACT, COMPARED AT INTERVALS AFTER TREATMENT WITH AVERAGE LENGTH OF COMPARABLE INTERNODES OF UNTREATED PLANTS

Comparison	No. days					Internode
	2	5	6	7	8	
Check	39	52	53	54	54	First
Treated	89	115	116	117	117	
Check	0	10	19	28	32	Second
Treated	0	99	111	120	120	
Check	0	0	0	0	10	Third
Treated	0	5	32	74	95	
Check	0	0	0	0	0	Fourth
Treated	0	0	0	23	57	
Check	0	0	0	0	0	Fifth
Treated	0	0	0	0	10	

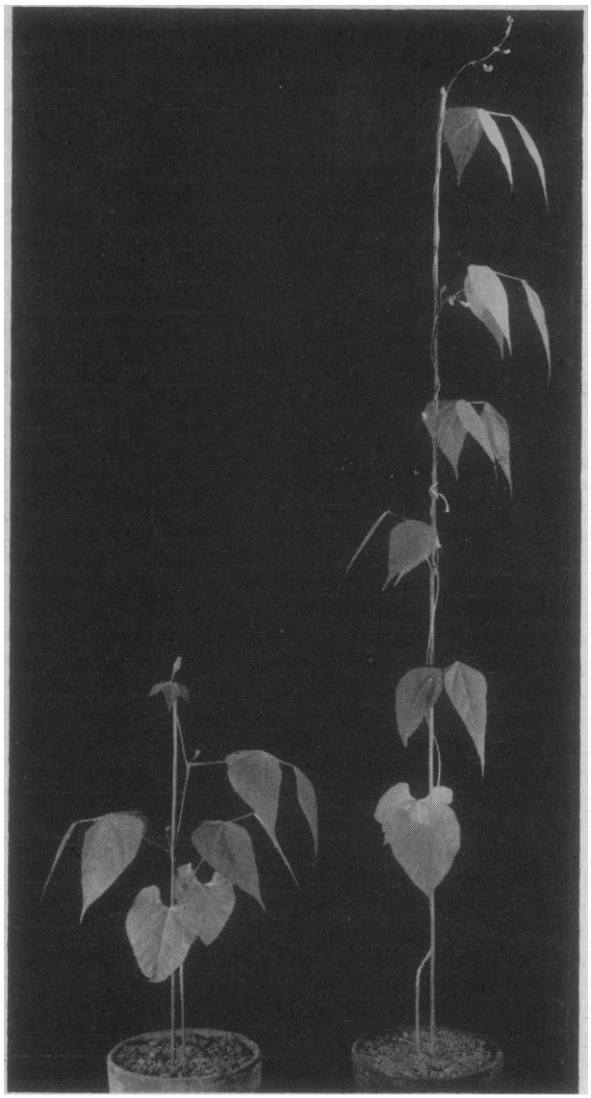

FIG. 2. Growth of bean plant (Black Valentine variety) following application of 125 μg hormone extract from immature seeds of other bean plants of the same variety, right. Comparable untreated plant, left. Photographed 3 weeks after treatment.

stems, but had no apparent effect when applied in equal amounts to the more mature part of their stems (hypocotyls) near the soil level. It failed to accelerate stem elongation of the seedlings when applied to either the upper or the lower surface of the primary leaves. Apparently hormones in the extract were not translocated from these leaves in sufficient amounts to bring about an observable effect in the stems.

Expansion of the primary leaves of seedlings was accelerated following application of the extract to a limited area on their upper or lower surfaces. Approximately 13 μg of the crude extract was applied in lanolin along the midribs of leaves, so as to cover an area of approximately 10 mm^2 on each leaf. The treated area was near the petiole attachment, in some instances on the ventral surface and in others on the dorsal surface. Elongation of the treated segment of midrib was accelerated. In addition, expansion of the treated leaves was increased so that their final area exceeded that of untreated ones by an average of 29.8% within a period of 6 days following treatment.

Untreated bean plants of the Black Valentine variety developed relatively short stems when grown under light supplied solely by means of daylight fluorescent tubes. In spite of the tendency to be short under these conditions, stems of seedlings treated with approximately 125 μg of the extract grew to be 3 times the height of untreated ones during the first 5 days of exposure to light from this source. Ten treated and 10 untreated plants were used in making the comparison, and the intensity of light used was 700–800 ft-c.

Since indolacetic acid accelerates cell elongation, the response of the plants to this compound was compared with that induced by means of the extract. Response to the extract was different in several respects from that which resulted from application of the acid. In comparing the responses, two series of treatments, comprised of 9 dosage levels of the acid and 9 of the extract, were prepared, with lanolin as a carrier. The dosages applied ranged from 125 to .013 μg/plant in each series. In treating, the paste was applied as a band around the first internode of each plant. Twenty-four hr after treatment, first internodes of plants treated with the optimum cell-elongating dose of the acid increased in length an average of 318% more than did those of untreated plants. During the same period, first internodes of seedlings treated with the most effective dose of seed extract increased in length an average of 870% more than did the untreated ones. Indolacetic acid brought about root initiation at the higher dosage levels, but the extract failed to cause this type of response at the highest dosage level used. The stimulating effect of the acid was confined to the treated first internode, whereas the extract not only accelerated elongation of this internode but also stimulated elongation of all internodes that developed above this segment of stem.

The extract had a systemic growth-stimulating effect when applied in minute amounts to the cotyledons of bean seeds that had just broken their coats in the process of germinating. Before the treatment was applied, the coats were removed from seeds that had developed radicles about 2.5 cm long. Approximately 13 μg of the extract was applied in lanolin as a thin layer covering about one fourth of the dorsal surface of each cotyledon. The treated seeds were replanted, along with an equal number of comparable but untreated ones from which the coats had also been removed.

Within 2½ days, growth of plants from treated seeds was noticeably more vigorous than that of the controls. This advantage persisted, and after 10 days the average lengths of the hypocotyls and first, second, and third internodes of plants that developed from the treated seeds were 10.9, 48.5, 65.8, and 100% greater, respectively, than the lengths of comparable

parts of plants from untreated seeds. The area of each leaf that developed during this period was increased by the seed treatment. Ten days after treatment, the plants from treated seeds had a total leaf area that was 27.6% greater than that of plants from untreated seeds.

Additional experiments are necessary to determine the effect of the seed extract on seed production of bean plants and to learn whether the growth of other crop plants, such as cereals, can be stimulated by phytohormones obtained from their seeds.

References

1. AVERY, G. S., JR., BERGER, J., and SHALUCHA, B. *Am. J. Botany*, **29**, 612, 765 (1942).
2. GUSTAFSON, F. G. *Ibid.*, **26**, 189 (1939).
3. HAAGEN-SMIT, A. J., *et al. Ibid.*, **33**, 118 (1946).
4. HATCHER, E. S. J. *Ann. Botany*, **9**, 235 (1945).
5. LUCKWILL, L. C. *J. Hort. Sci.*, **24**, 32 (1948).
6. WHITTWER, S. *Missouri Research Bull. No. 371* (1943).

Comments and Communications

Discriminatory Practices

ONE aspect of scientific life that seems to our department to require more general notice is that of eliminating discrimination against Negro scientists. Such discrimination occurs in many ways. Even when individual Negroes succeed in breaking through the barriers of economic, political, and academic discrimination sufficiently to acquire scientific training, they find employment opportunities severely restricted. In mathematics, at least, most qualified Negroes who find employment commensurate with their qualifications teach in Southern colleges. In attempting to participate in scientific life by way of the scientific societies, they become discouraged at the numerous restrictions placed on their participation and, in most instances, soon desist.

It is our view that the scientific societies, with their talk of the international character of science, must recognize its interracial character and put an end to discriminatory practices at meetings, etc. Accordingly, we have sent the attached letter to each member of the executive bodies of the two chief national mathematics organizations, and request you to publish it.

LEE LORCH

Department of Mathematics
Fisk University

DEPARTMENT OF MATHEMATICS,
FISK UNIVERSITY,
NASHVILLE, TENNESSEE
April 20, 1951

Executive Council, American Mathematical Society
Board of Governors, Mathematical Association of America

GENTLEMEN:

The Fisk Mathematics Department hereby requests the American Mathematical Society and the Mathematical Association of America to insert into the respective bylaws of the two organizations explicit and effective protection of the rights of all members to participate fully, freely and equally in the affairs of the organizations without regard to race, creed or color. It should be stated flatly that meetings, and teas, receptions, luncheons, dinners, etc., associated with meetings, shall be held only at institutions, hotels, etc., where prior assurance is given that there will be no discrimination. In keeping with such a policy, it should also be voted that persons holding office shall not participate in any way in any meeting of the organization until they have been assured of full compliance with the letter and spirit of this bylaw, failure in this respect to be construed as resignation from office.

The adoption of such bylaws is necessary for the protection of the rights of members and of the mathematical community generally, as the episode related below will establish. It is also necessary for the growth of membership and participation among Negro mathematicians, nearly all of whom teach in Southern schools and who will need such specific assurances before they will feel inclined to participate in the work of these organizations. It would follow the lead of other professional societies which have already codified this basic policy, for example, the American Psychological Association (*Am. Psychol.*, 5, 548 [1950]).

One of the events which demonstrate the need for such official action occurred in connection with the annual meeting of the southeastern region of the Association, March 16–17, 1951, with Peabody and Vanderbilt as hosts. The official program of the meeting announced a dinner at which the national President of the Association would be the speaker. The chairman of the Fisk department requested four reservations in a note handed personally, on March 10, to the individual specified on the announcement. On March 15, the day before the banquet, the chairman of the arrangements committee telephoned Fisk to inquire if any of the four reservations would be used by Negroes. On receiving an affirmative reply, he declared the reservations cancelled, stating that the arrangements committee would issue no tickets to Negroes.

Two of the undersigned, acting for the department, then requested the national President to act against this discrimination. He spoke to the arrangements committee chairman and then reported that the latter was determined to exclude Negroes. Thereupon our committee requested the President to withdraw from the dinner in order to avoid giving the impression that the national officers sanction or tolerate such discrimination or, if unwilling to withdraw, to make his disapproval of discrimination clear in his speech at the dinner. He felt that he could not do either, holding the view that such acts would be discourteous to his hosts.

Some further clarifying remarks may be in order. There is no state law or city ordinance which prohibits interracial dinners. Of this we have been assured by local attorneys with long experience in such matters. There is substantial precedent for such dinners, which have been held even in some local hotels, which are frequent in some churches and other semiprivate dining halls and which are daily events at Fisk.