ANATOMY OF PHASEOLUS VULGARIS ROOT TIPS AS INFLUENCED BY GIBBERELLINS

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By

RICHARD HUGH DELANO

AN ABSTRACT

Submitted to the College of Agriculture, Michigan State University
of Agriculture and Applied Science in partial fulfillment
of the requirements for the degree of

MASTER OF SCIENCE

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Approved 

RICHARD HUGH DELANO ABSTRACT

Phaseolus vulgaris 'Blue Lake' plants grown under greenhouse conditions were treated with 0, 10, 20, 100 and 500 micrograms of gibberellin. Treatment was made to determine the effect of gibberellin on the root and the site of its greatest activity.

Plants in one group were treated with 0, 10 and 20 micrograms of gibberellin applied dropwise in aqueous solution to the apex and harvested 72 hours later. Additional plants were treated similarly with 0 and 20 micrograms and harvested after 96 hours.

A subsequent group of plants was treated by saturating the medium with 0, 10 and 20 micrograms per milliliter of solution. Other plants received 0, 20, 100 and 500 micrograms of gibberellin applied dropwise to the shoot apices. All plants were harvested 72 hours after treatment.

Linear measurements were recorded and permanent slides prepared from longitudinal sections of the root apices. In addition, roots from plants treated with 0, 20, 100 and 500 micrograms of gibberellin were sectioned transversely and longitudinally 2, 30 and 60 mm from the root apex.

Neither concentration nor method of application affected the total length of the root. Root cortical cells 30 mm from the apex appeared to elongate more rapidly in treated plants without affecting the ultimate cell length. Maximum effects were obtained with 20 and 100 microgram applications, 20 being more effective.

Accompanied by six tables and three figures.

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INTRODUCTION

One of the most interesting groups of agricultural chemicals popularized in recent years has been the gibberellins. This material has been shown to have a varied effect upon many plants. A table compiled by Wittwer and Bukovac (1958) showed the effects of gibberellins on various plant parts as well as several physiological responses modified by them.

As gibberellins have become more readily available, the number of studies has increased proportionally. Responses of plant roots to gibberellins has not been clearly defined as most studies have been based primarily on plants grown in nutrient culture (Stowe and Yamaki, 1957). The determination of gibberellin effects on Phaseolus vulgaris roots, the sites of greatest activity and anatomical changes in the root were investigated.

LITERATURE REVIEW

Interest in the gibberellins was confined principally to Japan until 1950. In that year Mitchell and Angell introduced the gibberellins to the United States. About the same time gibberellins were brought to Europe by the Imperial Chemical Industries, Ltd. of Great Britain. A plethora of papers on the subject has appeared since that time.

Stem Elongation:

The most frequently noted phenomenon produced by the gibberellins was stem elongation. Some of the many examples were: Allium fistulosum, Kato (1955); Triticum vulgare, Brian and Grove (1956); Phaseolus vulgaris, Feucht (1957); Euonymus fortunei vegetus, McVey and Wittwer (1957); and Pisum sativa, Brian and Hemming (1957). The increase in stem length has been attributed to increased cellular division (Sachs and Lang, 1957; Feucht, 1957; and Greulach and Haesloop, 1958). Sachs and Lang (1957) reported that Hyoscyamus niger exhibited increased cellular division within twenty-four hours after treatment. Approximately equal anticlinal and periclinal divisions occurred in untreated plants. Treated plants showed primarily anticlinal divisions. Kato (1955), however, attributed stem elongation of Vegna sesquipedalis and some fern protonemata to cell elongation. Another

investigator, von Maltzahn (1957) found increased cellular length in the moss, Splachnum ampullaceum resulting from the use of gibberellin.

Dwarfing:

Gibberellins have overcome dwarfing in a number of plants. Genetically dwarf Pisum sativa grew like standard peas when treated with gibberellins, according to Brian and Grove (1957) and Bukovac and Wittwer (1956). The application of gibberellins caused dwarf Zea mays to assume the stature and appearance of normal plants in many cases (Brian and Hemming 1955). Extreme dwarfs manifested the greatest relative elongation in response to gibberellin treatment, whereas normal size plants responded only moderately. Gibberellins have been reported to eliminate physiological as well as genetic dwarfing (Barton 1957). The cold and light treatment required to overcome dwarfing of Malus arnoldiana 'Sargent', was replaced by gibberellins (Barton 1956).

Metabolic Response:

Kato (1956) reported increased oxygen uptake by pea stem sections grown in a medium containing gibberellin. An increase in metabolic rate was indicated by production of juvenile leaves in Hedra (Robbins 1957). Rappaport (1957) found that gibberellins induced juvenility in the tomato

leaf. In addition to juvenility, other changes were induced in the leaves by gibberellins. Saintpaulia ionathia produced longer leaf blades and petioles when treated with gibberellins (Roberts 1957). Kuraishi and Hashimoto (1957) observed that gibberellin treated discs of pea leaves increased in size, but not in fresh weight. This growth was attributed to cell expansion and not to cell division. Intact leaves showed no unusual expansion. Scott (1957) obtained similar results with bean leaf discs.

Plants in treated plots of Poa prathensis increased in both fresh and dry weight when used in combination with a 10-10-10 fertilizer (Leben and Barton, 1957; Wittwer and Bukovac, 1957). Morgan and Mees (1956) showed that the yield of a hay field treated with gibberellins decreased in dry weight at the first cutting. Later cuttings gave reduced yields. The increased weight of the first cutting equaled the weight lost in the second cutting.

Seed Activity:

Seed germination was influenced by gibberellins--Paeonia suffruticosa (Barton, Lela V., Fine and Chandler, 1957) and Malus arnoldiana 'Sargent' (Barton, Lela V., 1957). In these instances, vernalization requirements were supplanted. The red light requirement

for germination of Lactuca sativa 'Grand Rapids' seed was substituted by a mixture of gibberellins A_1 and A_3 (Kahn, 1957). Moore (1957) found no germination differences in treated and untreated Pisum sativa 'Telephone'. The gibberellins were imbibed through the seed coat. This was further confirmed by Wittwer and Bukovac (1957).

As with seed dormancy, gibberellins overcame the cold requirement for flowering in rutabaga 'Purpletop' (Bukovac and Wittwer, 1957), Matthiola incana and Viola tricolor (Lindstrom, Wittwer and Bukovac, 1957). Various concentrations of gibberellins were applied as foliar sprays. In addition, Wittwer and Bukovac (1957) found that several long-day annuals could be induced to flower under non-inductive conditions. Some examples were Lactuca sativa 'Grand Rapids' and Cichorium endiva (Endive).

Effect on Reproductive Parts:

Gibberellins induced fruit set and parthenocarpic fruit in tomato 'Earlypak' (Rappaport, 1957). Anthers of Saintpaulia ionantha, according to Roberts (1957), became broad as compared to non-treated plants. Lillium speciosum pollen failed to germinate without gibberellins, according to Chandler (1957). Several other varieties showed increased pollen germination and tube length when treated.

Heat induced "dormancy" in Lycopersicun esculentum fruit was overcome by gibberellin treatment. Gibberellins have reversed the "dormancy" of far red radiation (Liverman, 1957), but the normal size of the fruit was not increased (Rappaport, 1957).

Translocation:

Gibberellins are freely translocated. Peas grown in culture solutions containing gibberellins, or which received foliar application of gibberellins, gave comparable results (Brian, 1954; Bukovac and Wittwer, 1956; and Lippert, 1958). Potato plants sprayed before harvest and tubers after harvest exhibited sprouting. From these results it was assumed by Lippert (1958) that gibberellin movement was both acro and basipetal.

Gibberellins naturally occur in the higher plants, according to Phinney (1957). From seeds of Phaseolus vulgaris and Echinocystis he was able to isolate substances of a different chemical nature which produced growth patterns similar to those of gibberellin.

Root Growth:

The action of gibberellins on roots varied with the genus, species and variety. Brian (1957) stated that root growth of wheat seedlings was not stimulated by any known condition involving gibberellin. High concentrations of gibberellins may be inhibitory (Brian, 1957). Bukovac and

Wittwer (1957) reported root inhibition in Beta vulgaris. They observed (1956) that Phaseolus vulgaris 'Blue Lake' grown in solution culture exhibited no increased extension or dry weight increase of the roots. Field grown Zea mays treated with foliar sprays increased the size and number of anchor roots (Wittwer and Bukovac, 1957). Crude extracts of the fungus, Gibberella fujikuoi were made by Shimada (1932) and added to solution culture in which several types of plants were grown. The Azuke bean 'Maruba' (Phaseolus radiatus) responded with a markedly increased root growth. Zea mays 'Longfellow' and Glycine Max 'Akasaya', under the same conditions, failed to exhibit any such growth phenomena.

Whaley and Kephart (1957) cultured 10 mm apical sections of maize roots on moist filter paper containing gibberellins. Two inbred lines and their resulting progeny were used. One parent increased root length 24 percent when 20 micrograms per ml were used, while the other plant exhibited essentially no response. The progeny more nearly resembled the highly responsive parent. These findings agreed some what with those of Wittwer (1957) but were contrary to those of Shimada (1932).

Richardson (1958) has shown that light inhibits root growth in Pseudotsuga menziesii. Solutions of three ppm gibberellin stimulated

root growth in both light and darkness. Apical sections five to ten mm in length showed the greatest response of the three different lengths used. Phaseolus vulgaris (dwarf type) grown at high fertility levels manifested a decreased nodulization when gibberellins were sprayed on the leaves (Thurber et al., 1958).

Descriptions of the naturally occurring disease 'Bakanae' show various symptoms (Stowe and Yamaki, 1957). When the infections were not severe, attenuated root growth was observed in rice. More severe infections and possibly a higher concentration of gibberellins caused adventitious roots to form at the aerial nodes.

MATERIALS AND METHODS

Five seeds of Phaseolus vulgaris 'Blue Lake' were sown in each of twenty-five 6-inch clay pots containing terralite on February 10, 1958. The seeds were covered with one-half inch of terralite vermiculite medium grade, watered with distilled water, and placed in a 60° F night temperature greenhouse. Three seedlings were rogued from each pot. Plants were paired on the basis of leaf area and shoot length. Two plants of a pair were not necessarily in the same pot. One plant of each pair was treated and the other served as a comparison. Plants were treated in a random block pattern.

Aqueous solutions containing Tween-Twenty[1] and Gibrel[2] were used for treatment. A 2 cc syringe with a calibrated 24 gauge needle was used or application dropwise of the treating solution to the shoot apices. Seven plants were treated with 10 micrograms and seven with 20 micrograms of gibberellin. Seventy-two hours after treatment the plants were harvested, at which time linear measurements of the longest extension of the roots were recorded. Shoot length was measured from the second node to the apex.

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[1] A wetting agent manufactured by the Atlas Powder Company, Wilmington, Delaware.

[2] Potassium salt of gibberellic acid manufactured by Merck and Company, Inc., Rayway, New Jersey.

Severed shoot and root tips were placed in a solution of formalin-aceto-alcohol (FAA). Two fifteen minute aspiration periods were completed during the 48-hour fixing process. Tertiary-butyl alcohol series was used for dehydration. Specimens were embedded in histowax (53 to 56°), sectioned longitudinally at 10 microns, stained with safranin and fast green, and permanent slides prepared for microscopic examination of the medial sections. Average length of cells was determined near the apical cell and again approximately one-half mm basipetal to the apical cell.

A second series of forty pots of Phaseolus vulgaris 'Blue Lake' was planted on February 25, grown and paired as before. Twenty plants were treated on March 24 by saturating the medium with a gibberellin solution. A solution of ten micrograms per milliliter of aqueous solution was applied to one-half the plants, while the remainder was treated with 20 micrograms per milliliter of gibberellin solution. Collections were made 96 hours later. Sectioning, mounting and staining were performed as before.

A third series was planted on April 1, 1958, using the same methods. All plants were treated with a 20 micrograms per milliliter solution of gibberellins on April 18. Pots were plunged in the treating solution until

the medium was saturated. At the end of 72 hours the number of lateral roots and root and stem length on all plants was recorded. Shoot and root tips were killed and fixed in Randolph's modified Navashin solution. Material was embedded, sectioned, and stained as previously described for microscopic examination.

A final series of 48 pots was planted October 3, 1958 and grown under conditions previously described. Eight replicates were arranged according to shoot length and expansion of the first leaf. The plants were treated with 0, 20, 100, and 500 micrograms of an aqueous solution of gibberellins. The solution was applied to the stem apices with a calibrated needle. After 72 hours linear measurements of the shoot and root and the number of lateral roots were recorded.

Sections were killed and fixed in Randolph's modified Navashin's solution. Three to five root tips were harvested from each plant. Specimens 8 mm in length were collected from the root tip, and at points 30 and 60 mm from the tip. Permanent slides were prepared of longitudinal and transverse sections. Linear measurements were made of the cortical cells, 2, 30 and 60 mm from the apical cell and of epidermal cells 2 mm from the apical cell. Linear measurements of the roots were subjected to "Students t-test". An analysis of variance was performed on the cortical and epidermal cell length with respect to the various treatments. Significance was based on the multiple F test method of analysis (Duncan, 1955).

RESULTS

Treated bean plants produced elongated stems (Tables I, II and III) with somewhat etiolated leaves. Petioles exhibited similar characteristics. Leaves of treated plants were more expanded (Figure 1) than untreated plants. Roots of treated and non-treated plants on macroscopic inspection showed no apparent variation in diameters, fibrousness, color or number of lateral roots (Table IV). Gibberellins applied to the medium or stem apices failed to produce significant changes in the growth patterns (Tables I, II, III and IV). Treated roots harvested 72 and 96 hours after treatment were similar to those from untreated roots. Only slight differences were observed in the length of cortical cells at the apex or 60 mm basipetal to it (Table V). Cortical cells increased in length 30 mm from the apex after gibberellin treatment (Table V). Differences between untreated plants and those treated with 500 micrograms were not significant. Minor differences were observed between treatments of 100 and 500 micrograms, and between 20 and 100 micrograms. However, cortical cells from plants which received 20 or 100 micrograms of gibberellin were significantly longer than those of untreated plants. Plants treated with 20 micrograms of gibberellin showed the greater response.

TABLE I

The Length in mm of Roots of Phaseolus vulgaris 'Blue Lake' Treated with 10 and 20 Micrograms of Gibberellins Applied to the Apex and Harvested After 72 Hours (Average of 10 Roots).

Concentration Micrograms per Plant	Stem		Root		
	Untreated	Treated	Untreated	Treated	
10	101	226	283	243	N. S. *
20	111	213	252	255	N. S. *

*Student's t-test indicated no significant difference.

TABLE II

The Length in mm of Roots of Phaseolus vulgaris 'Blue Lake' Treated with 10 and 20 Micrograms per Milliliter of Aqueous Gibberellin Solution Applied to the Medium and Harvested 96 Hours Later (Average of 10 Roots).

Concentration in Micrograms per Milliliter	Stem		Root		
	Untreated	Treated	Untreated	Treated	
10	112	227	388	324	N. S. *
20	69	317	365	323	N. S. *

*Student's t-test indicated no significant difference.

TABLE III

Stem and Root Length of Phaseolus vulgaris 'Blue Lake' Grown in a Medium Saturated with 20 Micrograms per Milliliter of Gibberellin, and Harvested 72 Hours After Treatment (Average of Nine Plants).

Length in Millimeters			
Shoot		Root	
Untreated	Treated	Untreated	Treated
49	197	321	332 N. S.*

*Student's t-test showed no significant difference.

TABLE IV

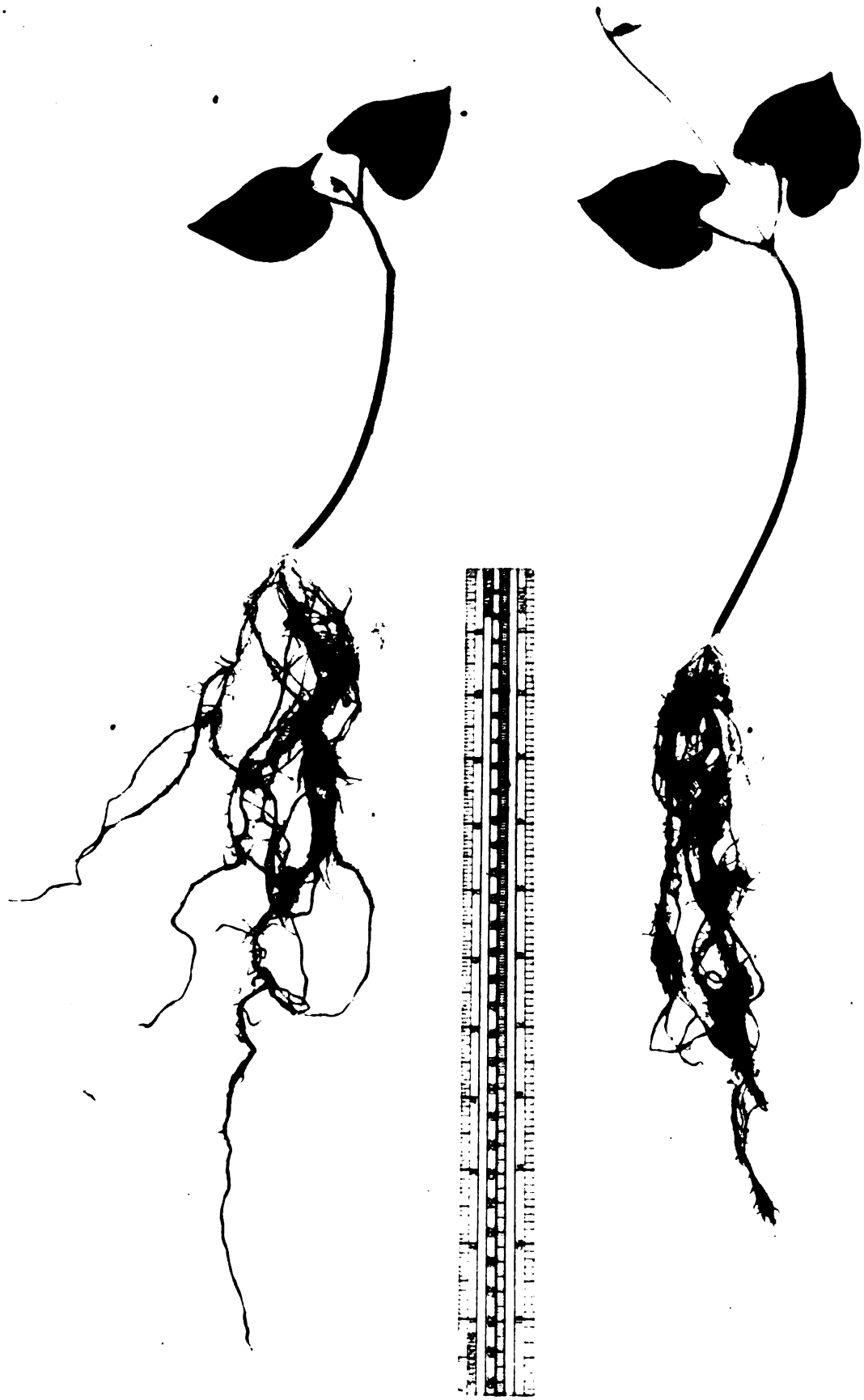
Root Lengths of Phaseolus vulgaris 'Blue Lake' Treated with Gibberellins Applied to Stem Apices and Harvested 72 Hours After Treatment (Average of Eight Plants).

	Concentration in Micrograms			
	0	20	100	500
Length (mm)	279	286	304	276
Number of lateral roots	9	10	9	9

Analysis of variance shows no significant differences.

Figure 1

Photograph of Phaseolus vulgaris 'Blue Lake' exhibiting the difference between treated and non-treated plants 72 hours after treatment.



Epidermal cells 2 mm basipetal to the apical cell did not respond to gibberellin treatment (Table VI). Transverse sections of root segments 60 mm from the apical cell showed a delayed rate of lignification when compared with untreated root sections (Figures 2 and 3). Higher concentrations of gibberellin appeared to inhibit lignification and to increase the size of intercellular spaces.

Studies of root tips harvested 72 hours after treatment showed that the majority of cell divisions were anticlinal. No significant differences in the number of anticlinal divisions were recorded for treated and untreated plants. One periclinal division occurred in an untreated plant. An average of six sections showed four divisions in the central cylinder, and ten in the cortex of untreated plants; three divisions in the central cylinder and eight in the cortex of those treated with 500 micrograms of gibberellins.

TABLE V

Influence of Various Concentrations of Gibberellins on the Length of Cortical Cells in the Root of Phaseolus vulgaris 'Blue Lake' at Various Distances from the Apical Cell (Average of 14 Cells). Gibberellins were Applied to Apices and Plants were Harvested 72 Hours Later.

Treatment (Micrograms per Plant)	Distance from Apical Cell		
	2 mm	30 mm (Length in microns)	60 mm
0	45	149	174
20	46	187*	184
100	44	174*	179
500	46	153	175
	N. S.	significant - see below	N. S.

	Micrograms		
0	500	100	20

A common line indicates no significance at 5%.

* Significantly different from control at 5%.

TABLE VI

Influence of Various Concentrations of Gibberellins. Epidermal Cell Lengths of Phaseolus vulgaris 'Blue Lake' Root Tips 2 mm from the Apical Cell. Gibberellins were Applied to Apices and Plants were Harvested 72 Hours Later.

Treatment (Micrograms per Plant)	Length in Microns (Average of Four Measurements)
0	48
20	50
100	47
500	47
	N. S. *

*Analysis of variance showed no significant difference.

Figure 2. Photomicrographs of longitudinal sections of Phaseolus vulgaris 'Blue Lake' roots 30 mm from the apical cell 72 hours after treatment. (a) Untreated; (b) treated with 20 micrograms gibberellin. Note lengthened cortical cells in treated sections. X 140.

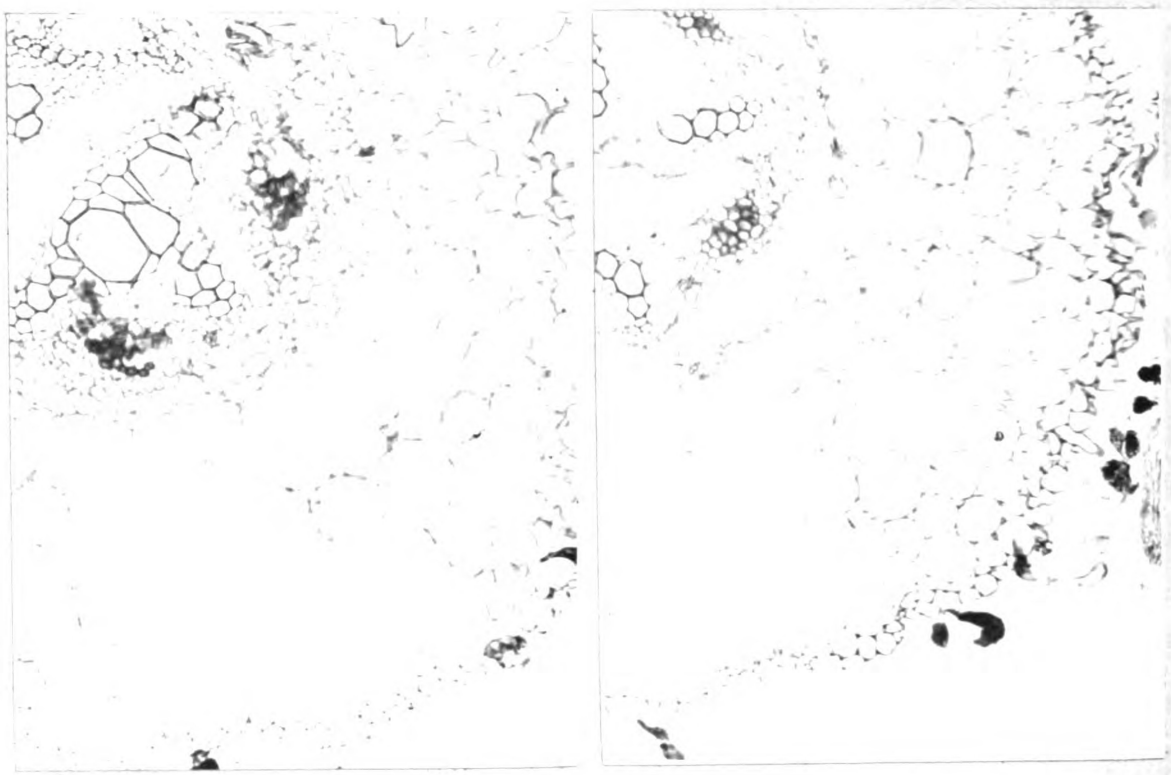


Figure 3. Photomicrographs of transverse sections of Phaseolus vulgaris 'Blue Lake' roots 30 mm from the apical cell 72 hours after treatment. (a) Untreated; (b) treated with 20 micrograms gibberellin. Note reduced lignification and slightly enlarged intercellular spaces in treated sections. X 140.

DISCUSSION

Gibberellin treatment failed to increase the number or vigor of the roots of Phaseolus vulgaris 'Blue Lake'. Injuries that could be attributed to treatment were not observed.

Differences in root elongation at any concentration and by any method of application to shoot tip or the medium were not found. These results were similar to those of Lippert (1958) who showed that gibberellins were freely translocated.

A comparison of the results shown in Tables II and III indicate that roots from untreated plants grow about 64 mm in 24 hours. Similar elongation did not occur during a comparable 24-hour period (72 to 96 hours after treatment) indicates an inhibition following treatment with 20 micrograms per milliliter of aqueous gibberellin solution. These results are confirmed by those of Brian (1957), who reported that in some instances gibberellins inhibited root growth. Apparently if inhibition is present, it extends for a longer time than the stimulatory effect inasmuch as Sachs and Lang (1957) reported an increased cellular division activity within 24 hours after treatment. In this instance inhibition or lack of stimulation remained for 72 and 96 hours after treatment.

The length of cortical cells was determined at the two, 30 and 60 mm levels. Cortical cells were measured because they were more uniform than those of the various tissues of the central cylinder. Epidermal cells were measured only at the two mm level because those at the 30 and 60 mm levels tended to slough. Only slight differences were observed in the epidermal cell length at any concentration. Cortical cells at the 2 mm level showed no difference at any concentration of gibberellin, but at the 30 mm level some response was found. Although the cortical cell at the 30 mm level elongated more rapidly in treated than untreated plants, the ultimate length of the cortical cell was not affected (Table V), suggesting that gibberellins influenced rate of growth and not cell length in the time between treatment and harvest. Morgan and Mees (1956) reported similar results from a study of grass shoots where the total length was apparently not affected by gibberellin application. Lower concentrations induced a greater response than higher concentrations of gibberellins.

Reduction in lignification and increase in size of intercellular spaces was accompanied by an increase in cellular length.

SUMMARY

Phaseolus vulgaris 'Blue Lake' plants grown under greenhouse conditions were treated with 0, 10, 20, 100 and 500 micrograms of gibberellin. Treatment was made to determine the effect of gibberellin on the root and the site of its greatest activity.

Plants in one group were treated with 0, 10 and 20 micrograms of gibberellin applied dropwise in aqueous solution to the apex and harvested 72 hours later. Additional plants were treated similarly with 0 and 20 micrograms and harvested after 96 hours.

A subsequent group of plants was treated by saturating the medium with 0, 10 and 20 micrograms per milliliter of solution. Other plants received 0, 20, 100 and 500 micrograms of gibberellin applied dropwise to the shoot apices. All plants were harvested 72 hours after treatment.

Linear measurements were recorded and permanent slides prepared from longitudinal sections of the root apices. In addition, roots from plants treated with 0, 20, 100 and 500 micrograms of gibberellin were sectioned transversely and longitudinally 2, 30 and 60 mm from the root apex.

Neither concentration nor method of application affected the total length of the root. Root cortical cells 30 mm from the apex appeared to elongate more rapidly in treated plants without affecting the ultimate cell length. Maximum effects were obtained with 20 and 100 microgram applications, 20 being more effective.

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