

SOME ANATOMICAL ASPECTS OF THE EFFECTS OF GIBBERELLIN ON PHASEOLUS VULGARIS

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SOME ANATOMICAL ASPECTS OF THE EFFECTS OF GIBBERELLIN

ON PHASEOLUS VULGARIS

By

JAMES ROGER FEUCHT

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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

Department of Horticulture

1957 P. Watson Approved

JAMES ROGER FEUCHT

ABSTRACT

Experiments were conducted to determine some of the anatomical effects of gibberellin on plants of <u>Phaseolus vulgaris</u> L. (cv. Blue Lakes) grown under greenhouse conditions.

Twenty micrograms of an aqueous solution of gibberellin were applied to the apical bud of <u>Phaseolus vulgaris</u> L. plants ten days after sowing. Growth measurements for treated and non-treated plants at the time of treatment and after 4, 8, 12, 24, 48, 72, and 96 hours, respectively, showed that response of gibberellin was at a good stage for study after 48 or 72 hours. These plants were prepared for microscopic examination and epidermal, cortical and pith cells of central longisections were measured in the longest dimension.

Additional plants were treated and prepared in the same manner as previously, eliminating all collections except those 48 and 72 hours after treatment.

Cells were counted in a single line of the pith in the first and third internodes of plants.

Cell number was shown to have increased in the internodes of all gibberellin-treated plants, but cell elongation accounted for the majority of the increase in plant height as a result of treatment with gibberellin.

Accompanied by five figures and six tables.

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INTRODUCTION

Recent introduction and the commercial availability of gibberellin into the United States have stimulated research investigations to determine the effects of gibberellin on plant growth and development. Results of research have captured the interest of chemical companies, commercial growers, and home gardeners. Despite the fact that there exists only a limited knowledge of their resulting effects upon plants, gibberellins are in demand and are being produced in quantity. Research continues at an increasing rate.

Current results have centered primarily around the chemical composition of the gibberellins (Curtis and Cross 1954, Stodola 1955, Borrow <u>et al.</u> 1955), the physiognomic characteristics produced as a result of gibberellin application on plants (Brian and Hemming 1955, Marth <u>et al.</u> 1956, Phinney 1956, Brian 1954, Bukovac and Wittwer 1956, Wittwer and Bukovac 1957, Kofranek and Phinney 1956), and to a lesser extent, the physiological and biochemical abberations of plants induced by gibberellins (Kato 1956, Lockhart 1956, Phinney 1956, Brian 1954). Anatomical effects that this chemical has produced on plants are not yet clearly demonstrated (Kato 1955, Sachs and Lang 1957).

The present investigation was designed to determine some of the anatomical effects of the gibberellins on <u>Phaseolus vulgaris</u> L. as an aid in a better interpretation of some plant growth characteristics produced by this chemical.

LITERATURE REVIEW

Origin

The gibberellins are crystalline compounds extracted from the culture filtrates of a fungus (<u>Gibberella fujikuroi</u>) which is responsible for the Bakanae disease in the Orient (Stodola 1955). This disease was first reported by Hori in 1898 on rice plants in Japan. Although the disease is found more commonly in rice, it also will infect corn (Stodola 1955).

Kurosawa (1926) demonstrated that the culture filtrates of the Bakanae fungus caused rice seedlings to elongate more than those not treated with the filtrate, and that the effect was the same as in plants actually infected with the fungus itself.

Brian <u>et al.</u> (1954) and Brian and Hemming (1955) in England, studied wheat and peas grown in water culture to which the substance was added. Their studies were concerned chiefly with the effects of gibberellin on dry and fresh weights, total nitrogen and carbon content of peas and wheat, and overcoming dwarfism of peas.

The first work with gibberellins in the United States was carried out by Mitchell and Angel in 1950 (Mitchell and Angel 1950). Stodola of the United States Department of Agriculture isolated a purified form of the substance in 1955.

Isolation and Chemical Composition

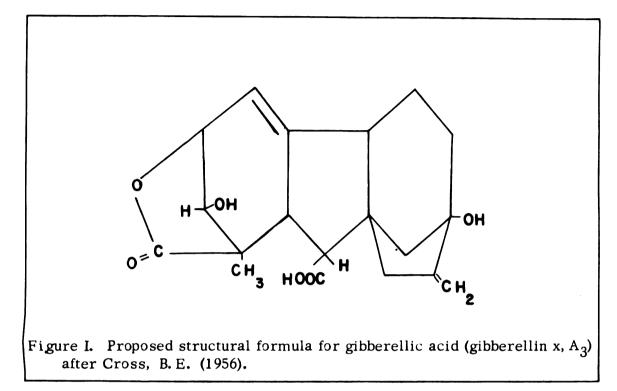
Since the original work of Kurosawa in 1926, Japanese, British and American scientists have been attempting to isolate the chemical that causes the growth phenomenon in plants, into its pure form.

Yabuta and Hayashi (1939) isolated a substance from Bakanae fungus which they called gibberellin A. Curtis and Cross (1954) isolated a crystallin compound they called gibberellic acid, which was later found by Cross (1954) to be different from the Japanese gibberellin A. Stodola (1955) isolated a pure crystallin substance from culture filtrates of the fungus which was found to be identical with the gibberellic acid isolated by Cross in 1954.

Recent attempts to identify and organize a new system of naming the compounds isolated by the Japanese and American scientists has resulted in the grouping of the compounds into three main classes, gibberellin A_1 ($C_{19}H_{24}O_6$), gibberellin A_2 ($C_{19}H_{26}O_6$), and gibberellin A_3 commonly known as gibberellic acid ($C_{19}H_{22}O_6$) (Stowe and Yamaki 1957).

Cross <u>et al.</u> (1956) worked out a tentative structure for gibberellic acid (Figure I).

Definite comparisons for the three substances have not yet been reported, however, the substances are known to have different melting points and rotation values (Cross 1954, Stodola 1955).



Despite differences in chemical and physical properties, Bukovac and Wittwer (1956) report no quantitative or qualitative differences in growth responses among gibberellin preparations. Similarly, Phinney (1956) found that both gibberellin A_1 and gibberellin A_3 stimulate plant growth.

Relation to Other Growth Regulators

A comparison of gibberellic acid with other growth regulators by Hayashi and Murakami (1954), using the <u>Avena</u> curvature test, shows that gibberellic acid was inactive in that test and that there was a marked difference in the mode of action between the gibberellins and auxins. Studies then followed by Hayashi and Murakami (1954), using the straight growth test of serial sections isolated from the base towards the apex of very young and vigorous leaves of cereal grasses. Gibberellic acid caused elongation of the basal sections, but was effective only in the presence of sugar. Indoleacetic acid, however, in-hibited growth of sucrose-suspended basal sections.

Phinney (1956a) treated mutant maize plants with indoleacetic acid, indoleacetic acid-nitrile, indolebutyric acid, 3-1 phenoxyacetic acid, 2, 4-dichlorophenoxyacetic acid, 2-naphthoxyacetic acid, alphanaphthalene acetic acid and gibberellin A_3 . He reported that mutants reversed to normal plants in the gibberellin treatments, but no effect was produced by the other growth regulators.

Effects on Plants

<u>Vegetative Growth</u>: The most pronounced effect of gibberellin on the growth of plant seedlings is the rapid elongation of the stem. Kato (1956), working with etiolated pea seedling sections, reported an increase of 76 per cent in length of the sections over the controls. Marth <u>et al.</u> (1956) found that cultivars of <u>Pelargonium</u>, <u>Poinsettia</u>, <u>Petunia</u>, <u>Callistephus</u>, and <u>Rosa</u>, when grown under greenhouse conditions, increased 50 to 300 per cent in height over the controls in a period of 3 to 4 weeks.

Bukovac and Wittwer (1956) and Wittwer and Bukovac (1957a) showed that many vegetables increased in the quantity of vegetation and that this increase was both in dry and fresh weights. Peas, beans and celery are a few examples of plants that increased in vegetative production after a treatment of 10 to 20 micrograms of gibberellin per plant.

Not all plants exhibit this vegetative response. Marth (1956) studied 49 different plant species and of these, <u>Gladiolus</u>, <u>Allium</u>, and <u>Pinus</u> showed no response to treatments using a 1 per cent lanolin paste containing concentrations of 1, 10, and 100 ppm of gibberellic acid.

Response of plants to gibberellin is not confined to normal-sized plants. Phinney (1956a), using seven genetically different dwarf mutants in maize, found that five of the seven dwarf cultivars responded to a treatment of gibberellin as low as 0.1 micrograms per plant, and that gibberellin caused a reversal of growth characteristics in the plants. Treated mutants became indistinguishable from normals. Phinney suggested that the response to gibberellins in dwarf maize was under genetical control and that the dwarfisms differed depending upon the mutation.

Brian and Hemming (1955) found that plants of the genetically dwarf pea, cv. Meteor, showed greater elongation when treated with gibberellin than did normal-sized peas. This work was later confirmed by Bukovac and Wittwer (1956) in a study using the Little Marvel cultivar of pea.

Lawn grasses, such as <u>Poa pratensis</u>, were induced into growth by treatments of very low concentrations of gibberellin long before non-treated grass showed signs of growth. Wittwer and Bukovac (1957b) treated replicated plots of <u>Poa</u> <u>pratensis</u> using aqueous solutions containing 10, 100 and 1000 ppm of gibberellin in early March in Michigan. No active growth was evident at the time of treatment, but within five days after treatment all treated plots showed signs of growth.

<u>Flowering and Fruiting</u>: In many plants the effects of treatment with gibberellin were not only exhibited in the foliage but also in the flowering behavior. Wittwer and Bukovac (1957a) found that head lettuce, broccoli, cabbage, leaf lettuce, beans, and many other vegetable crops grew more rapidly after treatment with aqueous solutions of gibberellin. These plants not only grew more rapidly but the length of time for flowering and fruiting was shortened. Some of the bean varieties flowered two to three days earlier and set pods a week earlier than the controls. Broccoli formed marketable heads ten days earlier in both greenhouse and field trials when sprayed with gibberellin. Head lettuce, cv. Great Lakes, flowered up to 30 days earlier and a greater percentage of the plants flowered and produced viable seed than did those not treated. Carrots, cabbage, and other biennials flowered without the usual cold requirement when treated at weekly intervals for a six-week period.

Lang (1956) reported that <u>Hyoscyamus</u>, <u>Anagallis</u>, <u>Dianthus</u> and <u>Silene</u> cultivars produced flower stalks under conditions that normally cause the plants to remain vegetative when treated with various rates of gibberellic acid.

Kofranek and Phinney (1956) treated <u>Callistephus</u> with the acid causing increased stem elongation and stimulated early flowering.

Additional studies have shown that gibberellic acid may retard flowering in some species of plants, but in others flowering may be hastened by one to several weeks (Marth 1956). He reported that long-day annuals, when grown under short days, will flower and produce seed when gibberellic acid is applied to the plants at the seedling stage of growth. Wittwer and Bukovac (1957a) treated lettuce, endive, radish, and mustard that was maintained under short days. The plants flowered and viable seed was produced. Flowering did not occur in the non-treated plants.

Effect on Seed Germination

Kahn and Goss (1957) demonstrated that seeds of Lactuca sativa L., requiring light for germination, may be induced to germinate in darkness when treated with gibberellin. They reported that gibberellin apparently replaces the primary red light requirement and prevents the secondary light requirement found in many seed lots of Lactuca sativa L.

Barton (1956) reported that seeds of <u>Malus</u> <u>arnoldiana</u> Sarg., when treated with gibberellic acid in a lanolin paste, would germinate and grow to normal-sized seedlings without the usual after-ripening treatment. Bredahl and Feucht (1956) found that excised embryos of <u>Malus pumila</u> <u>niedzwetzkyana</u> crosses elongated when placed on blotters moistened with 100 ppm gibberellin. Embryos placed on blotters moistened with distilled water showed no signs of elongation.

Anatomical Studies

In 1955 Kato (Kato 1955) studied the anatomical effects of gibberellic acid on seedlings of <u>Vigna sesquipedalis</u>, which differs from species of <u>Phaseolus</u> in that the keel is arched or curved inward rather than coiled and the flowers are usually few and somewhat capitate rather than racemose. Seedlings were treated when very young (3 to 4 cm tall) with 0. 1 per cent gibberellic acid in lanolin applied to the basal part of the cotyledons. Microscopic examination of the stems of his treated and non-treated plants showed no significant difference in diameter of cells, nor was there a significant difference in the number of cells per cross-section. He stated that "the most striking anatomical effect of the gibberellic acid treatment was the lower number of pith cells in a given longisectional area", and that...... "pith cells of the treated internodes were more than twice as large in this plane than were those of the controls". He suggested that stem elongation took place in the longitudinal rather than in the transverse direction and concluded that it was "..... the consequence of cell elongation rather than cell multiplication".

Sachs and Lang (1957) have shown that cell multiplication is significantly increased in the subapical region of non-vernalized but gibberellin-treated biennial <u>Hyoscyamus niger</u> rosettes, but no apparent effect was observed in the actual apical region of the plants. This phenomenon was in agreement with the hypothesis of Buvat who concluded that the most apical cells of the shoot of gymnosperms and three species of angiosperms did not have a histogenic or organogenetic role during vegetative growth (Buvat 1952). Sachs and Lang (1957), however, did not exclude the apex as a source of cell initiation.

PROCEDURE

Experiment One

A preliminary investigation was performed to determine, (1) the length of time required to obtain responses that were suitable for study, and (2) which tissues were most affected by the gibberellins.

On January 8, 1957, seeds of <u>Phaseolus vulgaris L.</u>, cv. Blue Lakes, were sown in 8-inch plastic pots containing clean quartz #7 sand, and were watered with distilled water. The pots were placed in the greenhouse where temperatures averaged 70° F during the day, and 60° F at night. Ten days after planting, the seedlings were assigned numbers by random selection. Each first internode was measured from the cotyledon axil to the base of the apical bud; these values were recorded. Plants designated as treated, were correspondingly treated with 20 micrograms of a mixture of gibberellin A_1 and gibberellin A_3^{-1} prepared as an aqueous solution², and applied with a micropipette to the apical bud of each plant. A 20 lamba drop of distilled water³ was applied to the apical bud of those plants designated for comparison.

^lGibberellin A₁ and gibberellin A₃ mixture supplied by F.H. Stodola, Northern Regional Research Laboratories, U.S.D.A., Peoria, Illinois.

2 The aqueous solution was made by dissolving 10 mg of the crystallin gibberellin mixture in 0.5 ml of 95 per cent ethyl alcohol and adding 9.5 ml of distilled water.

Prepared by adding 0.5 ml of 95 per cent ethyl alcohol to 9.5 ml of distilled water. After 4, 8, 12, 24, 48, 72 and 96 hours, respectively, three treated and three non-treated plants were measured in the same manner as before treatment. All plant parts immediately proximal to the cotyledons were cut from the plant.

The stems of plants 48 and 72 hours after treatment were killed and fixed in F.A.A. (90 parts of 70 per cent ethyl alcohol, 5 parts of formalin, and 5 parts of glacial acetic acid), dehydrated in tertiary butyl alcohol and embedded in Histowax (54° to 56°C). Longitudinal sections of the first internode were cut 10 micra in thickness with a rotary microtome, stained in safranin and fast green, and permanently mounted in piccolyte¹.

The preparations were examined to determine which cells or tissues exhibited the greatest effect after treatment, and in which tissue cells would be most conveniently observed for future studies. Measurements were made of the longest dimension of 10 epidermal, 10 cortical, and 10 pith cells located in approximately similar tissue in both treated and non-treated stems. These values were recorded and the ratios of sizes between the epidermal, cortical and pith cells were calculated. A total of 175 microscope slides were prepared.

^A dry resin product of General Biological Supply House Inc., Chicago, Illinois.

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Experiment Two

On January 21, 1957, plants of <u>Phaseolus vulgaris</u> L., cv. Blue Lakes, were grown in the same manner and under similar conditions as in Experiment one.

Ten days after sowing, 40 seedlings were assigned numbers by random selection. Each first internode from the cotyledon axil to the point of attachment of the petiole of the simple leaf and similarly, each second internode was measured and all measurements were recorded. Immediately after each measurement, 20 micrograms of Gibrel¹ was applied to the apical bud of 20 plants, and a 20 lambda drop of distilled water was applied to the apical bud of 20 other plants.

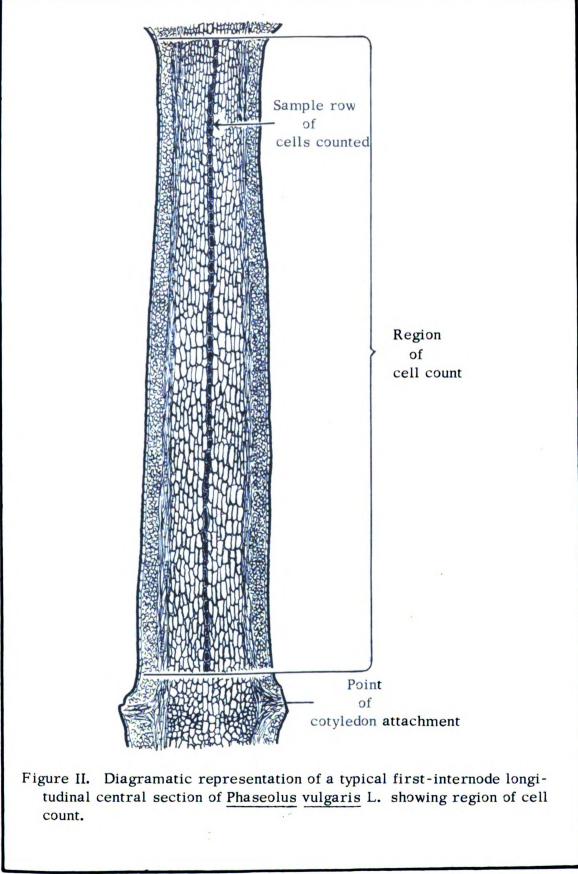
First and second internodes of ten plants of the treated and non-treated series 48 and 72 hours after treatment were measured, then cut from the plant and prepared for microscopic examination as before.

The number of cells of the first internode on a line in a single plane of the pith were counted as indicated in Figure II, and the values obtained were recorded. Identical counts were made of 10 serial sections per plant.

A "t-test" for unpaired plants (Goulden 1952) was performed to determine if the differences in average cell number were significant between treated and non-treated plants. A total of 156 microscope slides were prepared.

_ _ _ _ _ _ _ _ _ _ _

Brand name of the potassium salt of gibberellic acid produced by Merck and Company, Inc., Chemical Division, Rahway, New Jersey.

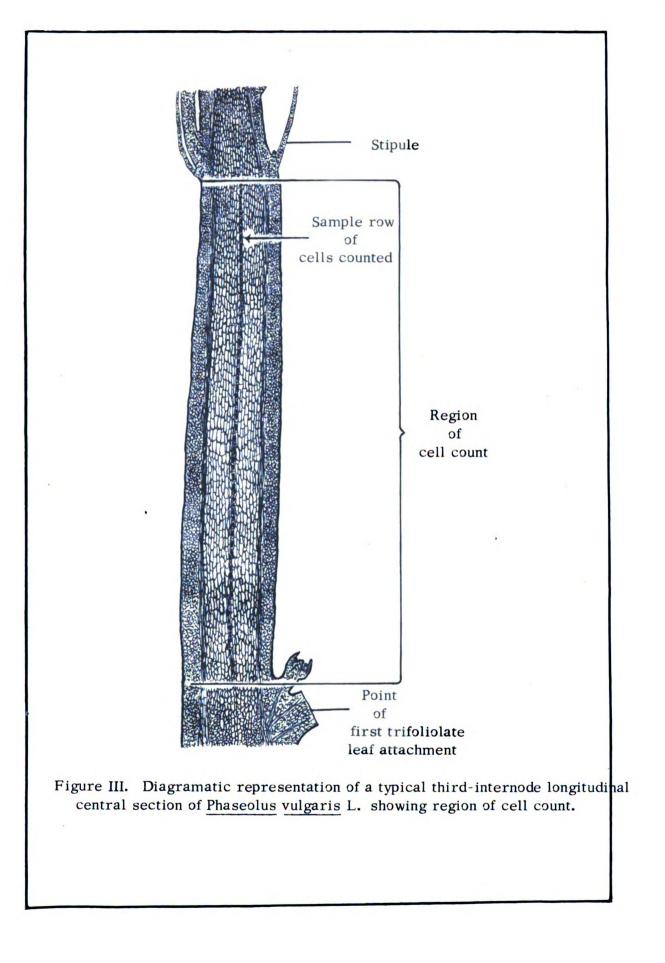


Experiment Three

On May 26, 1957, a third series of beans using seeds from the same source, was grown in a sterilized potting mixture (1/4 part sand, 1/4 part peat, and 1/2 part soil). The soil mixture was used at this time because of difficulties encountered with high temperatures in the quartz medium causing death in many of the plants.

Nine days after sowing, the seedlings were measured and paired. A total of 20 pairs were selected, each pair having closely similar internodal lengths. One of each of the pairs was treated with 20 micrograms of Gibrel in the same manner as before. After 72 hours the first, second and third internodes were measured, collected and processed, as before. Because of the higher room temperatures at this time of year, this series was embedded in Histowax 60° to 63°C. Ten of the third-internode pairs were cut and processed as previously.

Cells of the pith were counted on a single line beginning at the axil of the first trifoliolate leaf and extending to the base of the stipules of the terminal bud (Figure III). A "t-test" for paired plants (Goulden 1952) was calculated to determine the significant difference of average cell numbers in the third internode of treated and non-treated plants. A total of 110 microscope slides were prepared.



RESULTS

Experiment One

Preliminary experiments showed that plants of <u>Phaseolus vulgaris</u> L., when treated with gibberellin, increased in internodal length within 8 hours after treatment and the changes were at an optimum stage for cellular examination after 48 and 72 hours (Table 1). The response obtained in plants 96 hours after treatment was greater, but not sufficiently better than that at 48 and 72 hours (Table 1). Furthermore, the specimens collected 72 hours after treatment provided more readily manageable preparations.

Externally, treated plants differed in appearance from non-treated plants by the longer internodes (Figure IV). No other physiognomic changes were detected.

General observations of longitudinal sections of the first internode revealed that cells elongated more in treated plants than in non-treated plants (Figure V).

Measurements of epidermal, cortical and pith cells of treated and non-treated plants are recorded in Table 2. The average longest dimension of epidermal, cortical and pith cells from treated plants was 90.0, 49.3, and 446.1 micra respectively, and 45.0, 26.9, and 153.8 micra respectively, in non-treated plants. The ratio between epidermal, cortical and pith cells

| Treatment | | | Hours | Hours After Treatment | eatment | | | | Average In- |
|--|------|------|-------|-----------------------|---------|------|-------|-------|-------------|
| | 0 | 4 | ø | 12 | 24 | 48 | 72 | 96 | crease (Mm) |
| 20 Micrograms | 13.0 | 14.0 | | | | | | | 1.0 |
| of | 14.3 | | 16.0 | | | | | | 1.7 |
| Gibberellin | 8.6 | | | 13.3 | | | | | 4.7 |
| | 11.0 | | | | 22.6 | | | | 11.6 |
| | 8.6 | | | | | 34.0 | | | 26.4 |
| | 11.6 | | | | | | 32. 3 | | 20.7 |
| | 7.6 | | | | | | | 42. 6 | 35.0 |
| 20 Lambda | 5.6 | 8.0 | | | | | | | 2.4 |
| of | 13.0 | | 13.3 | | | | | | 0.3 |
| Distilled | 8.6 | | | 12.0 | | | | | 3.4 |
| Water | 14.0 | | | | 19.0 | | | | 5.0 |
| | 8.3 | | | | | 17.0 | | | 8.7 |
| | 11.6 | | | | | | 20.6 | | 9.0 |
| | 8.6 | | | | | | | 23. 3 | 15.3 |
| Average Increase of Treated Plants Over the Non- Treated (Mm) | | 0.0 | 1.4 | 1.3 | 6.6 | 16.7 | 11.7 | 19. 7 | |

Linear Growth (Mm) of First Internodes of Treated and Non-Treated Plants (Average of Three Plants)

TABLE 1

18

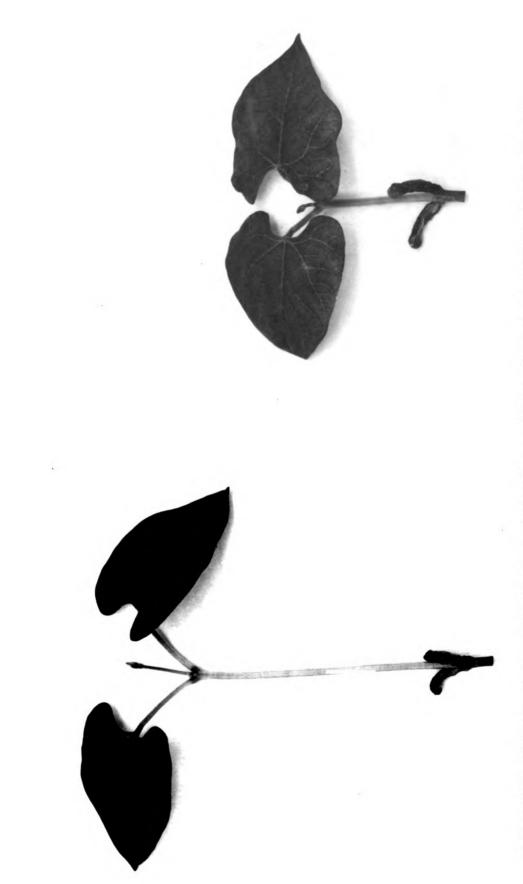


Figure IV. Phaseolus vulgaris L. plants cut below the cotyledons 72 hours after treatment with 20 micro-grams gibberellin (left), and 20 lambda distilled water (right). (Actual size).

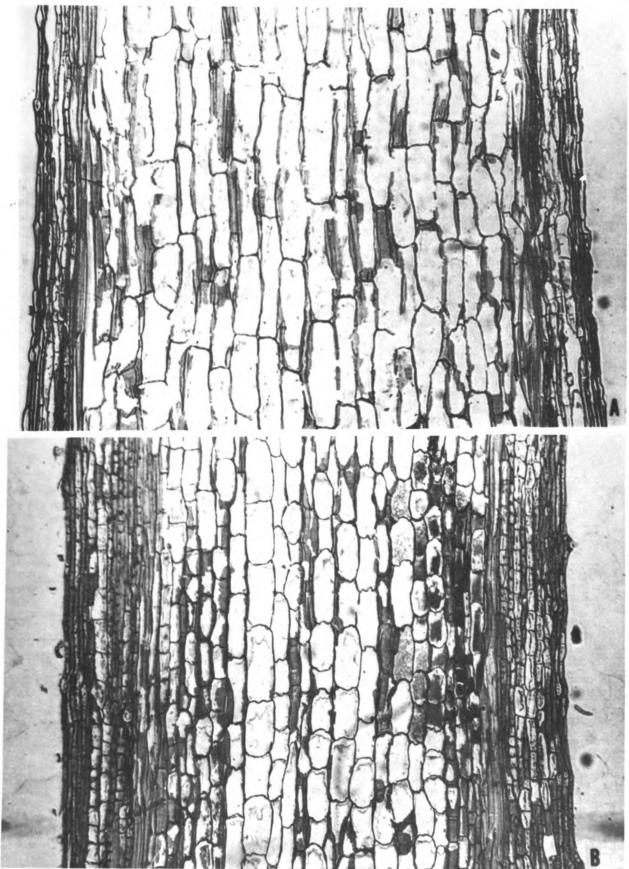


Figure V. Photomicrographs of longisectional area in the first internode of Phaseolus vulgaris L. plants 72 hours after treatment with (a) 20 micrograms gibberellin, and (b) 20 lambda distilled water. X 130.

| TABLE | 2 |
|-------|---|

| | | (Mi | crons) | | | |
|-------------|-----------|-----------|-------------|----------------|----------|--------------|
| Cell Number | 20 micro | ograms Gi | brel | 2 0 lam | bda Wate | r |
| | Epidermis | s Cortex | Pith | Epidermis | Cortex | Pith |
| 1 | 166 | 46 | 570 | 93 | 30 | 150 |
| 2 | 66 | 40 | 466 | 43 | 30 | 213 |
| 3 | 73 | 46 | 473 | 46 | 33 | 193 |
| 4 | 86 | 46 | 660 | 20 | 26 | 176 |
| 5 | 90 | 53 | 636 | 23 | 20 | 166 |
| 6 | 70 | 80 | 413 | 46 | 20 | 153 |
| 7 | 66 | 30 | 450 | 73 | 30 | 133 |
| 9 | 63 | 63 | 466 | 36 | 23 | 146 |
| 10 | 110 | 46 | 460 | 40 | 20 | 233 |
| 11 | 73 | 33 | 336 | 50 | 33 | 203 |
| 12 | 53 | 36 | 5 26 | 46 | 40 | 140 |
| 13 | 103 | 43 | 243 | 20 | 16 | 126 |
| 14 | 50 | 30 | 330 | 23 | 26 | 143 |
| 15 | 103 | 33 | 203 | 23 | 33 | 110 |
| 16 | 106 | 83 | 443 | 26 | 30 | 200 |
| 17 | 136 | 60 | 603 | 40 | 26 | 106 |
| 18 | 156 | 63 | 403 | 76 | 26 | 116 |
| 19 | 73 | 30 | 360 | 60 | 30 | 133 |
| 20 | 73 | 53 | 246 | 50 | 30 | 1 2 0 |
| Average | 90* | 49.3* | 446.1* | 45 | 26.9 | 153.8 |

Measurements of Cells in Longest Dimension (72 Hours after Treatment) (Microns)

*Differences significant at the 1% level.

in treated plants was approximately 2:1:9 and in non-treated plants the ratio was approximately 2:1:6.

After comparing measurements of these cells, pith cells were selected for cell-count studies because their size facilitated accuracy of observation. Experiment Two

An average of ten plants showed that the first internodes of treated plants increased 22.3 mm in linear growth, and the non-treated plants, 10.5 mm, after 48 hours (Table 3). Likewise, treated plants allowed to grow for 72 hours increased 21.55 mm and non-treated plants increased 11.4 mm (Table 3).

TABLE 3

| Treatment | Time (Hours) | Length of Int (average | Difference | |
|-------------------------|-----------------|---------------------------|--------------------|--------------|
| | (110010) | Before Treatment | After Treatment | |
| 20 micrograms Gibrel | 48 | 14.2 | 36.5 | 22. 3 |
| 20 lambda Water | 48 | 14.8 | 25.3 | 10.5 |
| 20 micrograms Gibrel | 72 | 15.9 | 37.4 | 21.5 |
| 20 lambda Water | 72 | 1 2. 0 | 23.4 | 11.4 |

Influence of Gibberellin on Length of Internode

The increases in linear growth of the internode as a result of treatment averaged 11.8 mm after 48 hours, and 10.1 mm after 72 hours. This difference of 1.7 mm was not found to be significant, and consequently only plants 72 hours after treatment were selected for cell counts.

The number of pith cells on a single line of the first internode (Figure II) averaged 185.2 in treated plants, and 152.1 in non-treated plants (significant at the 1 per cent level) (Table 4).

Experiment Three

Beans grown later in the spring in soil, when treated with gibberellin, showed greater response than those previously grown in sand (Tables 3 and 5). A third internode was visible in all of the plants 72 hours after treatment which, at the time of treatment, was enclosed within the terminal bud. Third internodes averaged 5.0 mm in length in treated plants, and 2.8 mm on the average in non-treated plants (Table 5). The greatest response to treatment with gibberellin was found in the second internode (Table 5). Second internodes, however, were discarded for cell count studies because of their large size which would have made processing difficult.

The number of pith cells in a single line between nodes of the third internode (Figure III) averaged 215.4 in treated plants, and 130.3 in non-treated plants (significant at the 1 per cent level) (Table 6).

| TA | BL | Æ | 4 |
|----|----|---|---|
|----|----|---|---|

Number of Pith Cells of First Internode (Counted as Shown in Figure II).

| 20 microg | rams Gibrel | 20 lambda Distilled Water | | | | |
|-------------------------------|--------------------------------------|-------------------------------|--------------------------------------|--|--|--|
| Length after 72 hours (mm) | Cell number (Average 10 Sections) | Length after 72 hours (mm) | Cell number (Average 10 Sections) | | | |
| 64 | 216. 9 | 23 | 140.4 | | | |
| 37 | 174.0 | 21 | 170 . 9 | | | |
| 38 | 149. 1 | 29 | 158.3 | | | |
| 32 | 175. 1 | 2 5 | 196.7 | | | |
| 33 | 165.8 | 16 | 140.5 | | | |
| 26 | 199. 9 | 28 | 138.3 | | | |
| 44 | 220. 8 | 22 | 134. 3 | | | |
| 40 | 185.9 | 26 | 146. 2 | | | |
| 30 | 170. 3 | 26 | 15 2. 9 | | | |
| 33 | 194.0 | 18 | 134.1 | | | |
| ve. 37.7 | 185. 1* | 23. 4 | 152. 1 | | | |

*Difference significant at the 1% level.

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Length of Internodes before Treatment and after 72 Hours (Average of 20 Plants in Millimeters)

| | First Internode | ernode | Difference | Second Internode | ernode | Difference | Third Internode | rnode |
|-----------------|------------------------------------|-------------------|------------|---------------------|-------------------|------------|---------------------|-------------------|
| Treatment | Before After Treatment 72 Hours | After 72 Hours | | Before Treatment | After 72 Hours | | Before Treatment | After 72 Hours |
| 20 micrograms | | | | | | | | |
| Gibrel | 13.8 | 39.6 | 25.8 | 4.6 | 43. 9 | 39. 2 | 1 | 5.0 |
| | | | | | | | | |
| 20 lambda | | | | | | | | |
| Distilled water | 13.8 | 24. 3 | 10.5 | 4.6 | 22.7 | 10.0 | | 2.8 |
| | | | | | | | | |

| 20 micrograms Gibrel | | 20 lambda Distilled Water | |
|-------------------------------|--------------------------------------|-------------------------------|-------------------------------------|
| Length after 72 hours (mm) | Cell number (Average 10 Sections) | Length after 72 hours (mm) | Cell number (Average 10 Sections |
| 7.0 | 238 | 4.0 | 157 |
| 4.0 | 244 | 3.0 | 176 |
| 5.0 | 131 | 2.0 | 118 |
| 5.0 | 195 | 2.0 | 57 |
| 5.0 | 235 | 2. 0 | 105 |
| 7.0 | 287 | 4.0 | 147 |
| 5.0 | 190 | 3. 0 | 143 |
| 4.0 | 162 | 1.0 | 70 |
| 7.0 | _ 267 | 3.0 | 137 |
| 5.0 | 205 | 5.0 | 193 |
| Ave. 5.4 | 215. 4* | 3.0 | 130. 3 |

Number of Pith Cells of Third Internode (Counted as Shown in Figure III)

TABLE 6

*Difference significant at the 1% level.

CONCLUSIONS

Data and observations from this study showed that gibberellin-treated plants of <u>Phaseolus vulgaris</u> L. not only increased in length of internodes to a greater extent than did non-treated plants, but also, in a linear direction, increased in cell number. This is in agreement with Sachs and Lang (Sachs and Lang 1957) who studied the effects of gibberellin on <u>Hyoscyamus niger</u> seedlings, but in direct disagreement with the report of Kato (Kato 1955) on the anatomical effects of 1 cm central sections of gibberellin-treated <u>Vigna</u> sesquipedalis plants.

Cell counts were made only in the pith of the plant internodes, however, measurements of epidermal and cortical cells in the longest dimension of both treated and non-treated plants indicated that these cells were proportional in number of those of the pith (Experiment One). It is conceivable, therefore, that all tissues were affected by the gibberellin treatments with an increase in cell division as well as cell elongation.

Although cell numbers are increased in the internodes of treated plants, cell elongation is responsible for the majority of the increase in plant height.

SUMMARY

Experiments were conducted to determine some of the anatomical effects of gibberellin on plants of <u>Phaseolus vulgaris</u> L. cv. Blue Lakes grown under greenhouse conditions.

A twenty microgram aliquot of an aqueous solution of gibberellin was applied to the apical bud of <u>Phaseolus vulgaris</u> L. plants ten days after sowing. Growth measurements for treated and non-treated plants at the time of treatment and after 4, 8, 12, 24, 48, 72 and 96 hours, respectively, showed that response to gibberellin was at a good stage for study after 48 and 72 hours. These plants were prepared for microscopic examination and epidermal, cortical and pith cells of central longisections were measured.

Additional plants were treated and prepared in the same manner as previously, eliminating all collections except those 48 and 72 hours after treatment.

Cells were counted in a single line of the pith in the first and third internodes of plants.

Cell number was shown to have increased in the internodes of all treated plants, but cell elongation accounted for the majority of the increase in plant height as a result of treatment with gibberellin.

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