ENVIRONMENTAL, GENETIC, AND DEVELOPMENTAL STUDIES OF THE HYPOCOTYL ELONGATION TRAIT IN PHASEOLUS VULGARIS (VAR. 211-V AND SANILAC)

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ENVIRONMENTAL, GENETIC, AND DEVELOPMENTAL STUDIES OF THE HYPOCOTYL ELONGATION TRAIT IN PHASEOLUS VULGARIS

(Var. 211-V and Sanilac)

By

ANTONIO M. PINCHINAT

AN ABSTRACT

Submitted to the College of Agriculture of 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 M. W. Adams

Antonio M. Pinchinat

ABSTRACT

To investigate on the differential hypocotyl elongation of two commercially grown varieties of Navy beans, environmental, genetic, and developmental studies were carried out.

Following application of alternative combinations of day length and light intensity treatments upon seedlings of Sanilac and 211-V from germination to complete development, it has been noticed that, although differences in average hypocotyl length between the bush type, Sanilac, and the viny type, 211-V, were more pronounced under long day, high light conditions than under any other treatment, difference in hypocotyl elongation was primarily attributable to intrinsic factors rather than to environmental influences.

To study the mode of inheritance of the short hypocotyl trait in Sanilac and its opposite, the long hypocotyl character in 211-V, appropriate crosses and backcrosses between the parent materials and their F_1 progeny were performed. Common beans (Phaseolus vulgaris) being a naturally self-pollinated crop, F_2 seedlings were obtained by growing seeds harvested from F_1 plants. Analyses of data gathered for this study suggest that transmission of the hypocotyl elongation trait in Navy bean seedlings could be interpreted on the basis of a duplicate recessive epistasis mode of inheritance in which the long hypocotyl trait would be caused by recessivity in two different genes, both in dominant condition being necessary to prevent the length growth of the hypocotylary axis.

Observations made on cortical cell layers of hypocotyl sections of the seedlings of Sanilac and 211-V disclose no appreciable difference in average cell length of both varieties, implying, therefore, the occurrence of a larger number of cells in a given hypocotylary cortical strand of 211-V as compared to a corresponding strand in Sanilac. Referring to recent studies on phytohormones and their relation to growth and development, it has been assumed that gibberellic or gibberellin-like sybstances might be taken into account for the marked increase in cell division rate of 211-V, the long hypocotyl variety. The results from the genetic and developmental studies, considered together, may suggest the following alternative hypotheses:

1. All bean varieties possess gibberellin-like substances which tend to promote hypocotyl elongation, but most varieties, like Sanilac, also possess a genetic inhibitory system which, when two non-allelic genes are present in dominant condition effectively suppress the phytohormonal growth action.

2. Or, only those varieties of beans with recessive alleles in the homozygous state at both the postulated loci involved are able to produce the gibberellin-like material and no direct inhibiting system is present.

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*BC_s: Back cross of F_1 to Sanilac.

**BC_v: Back cross of F_1 to 211-V.

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INTRODUCTION

Extensive and intensive studies have been carried out to find ways to develop varieties that are high in yield and quality, resistant to the most economically important plant diseases, and more fitted to modern crop management purposes and practices.

The present paper will not endeavor to set forth a list of the tremendous and innumerable advances which have been realized in field bean breeding and improvement in North America or abroad during the past half century. It is merely a modest contribution to the study of an important agronomic character of two commercially grown varieties of Navy pea beans (*Phaseolus vulgaris*) 211-V and Sanilac, which have directly or indirectly arisen from this invaluable crop breeding and improvement program.

Released by a private plant breeder, 211-V is apparently of mutational origin from the parental variety 211, which is declared to result from a cross of Robust x Black African. This new strain of Navy pea bean is a late maturing vine type, susceptible to the common bean mosaic, and characterized by its unusually elongated hypocotyl and basal internodes. (1)

The development of Sanilac has been briefly sketched by Down and Andersen (3). In 1940, an early bush mutant segregated from a lot of Michelite bean seeds treated with x-rays in 1938. This mutant was increased in size by back crossing to Michelite followed by selection. The recovered mutant strain was used in a cooperative bean breeding project begun in 1948 between the Michigan Agricultural Experiment Station (MAES) and the United States Department of Agriculture (USDA). This research program has resulted in the introduction of the bush type variety Sanilac to Michigan agriculture in 1956. Besides similarity with Michelite in seed type, good canning quality, and resistance to common bean mosaic (V-I), Sanilac is superior to its parent in a six day earliness and resistance to the α and β strains of the fungus causing anthracnose. Being an erect bush type, it holds its upper pods off the ground at harvest and offers mechanical resistance to white mold disease of beans. It conspicuously differs from the 211-V by its short hypocotyl section. (Plate I)

Considering this last point, in spite of outstanding agronomic features and its high position in the canning trade (18) further improvement is being viewed in Sanilac to bring its basal pods higher above ground level through appropriate breeding techniques. Success in this respect would result in the possibility of harvesting this crop by combine without pulling and with the least damage to the lower pods, and in a significant increase in its yield by decreasing losses due to the cull material or "pick."

In the present investigation an attempt has been made to study hypocotyl elongation of Sanilac and 211-V under different artificial environmental conditions, and to find an explanation to the inheritance of this agronomic trait by means of hybridization. Consideration also has been given to cell division and cell expansion in the above-mentioned region of seedlings of both strains of Navy pea beans.

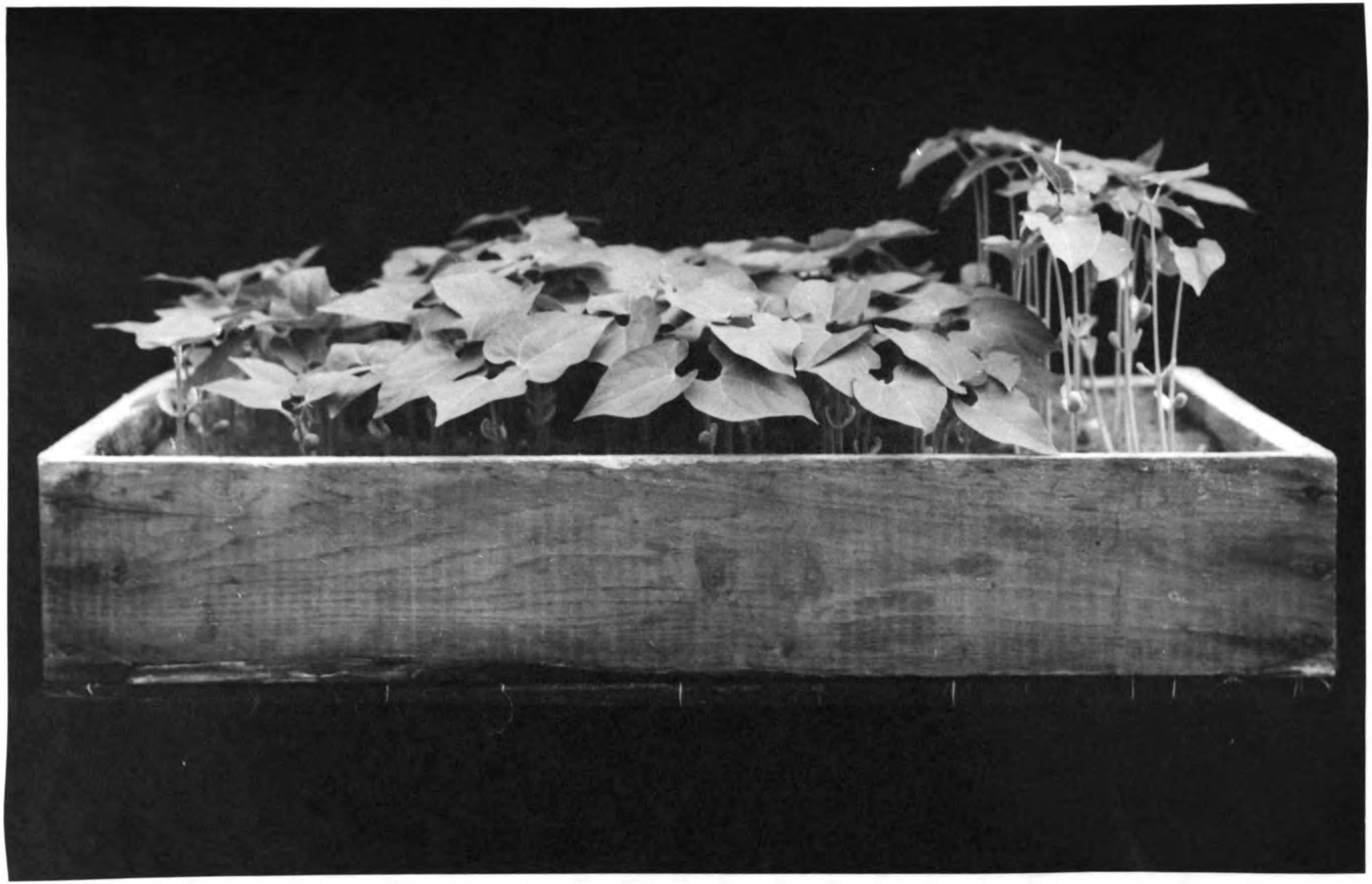


PLATE I. Young seedlings of Sanilac and 211-V.

REVIEW OF LITERATURE

As beans form an extremely important agricultural and horticultural crop plant all over the world, the existence of an extensive literature on the subject is quite natural. To insure optimum conditions for growth, increase yield, and overcome disease hazards, particular consideration has been devoted to the study of environmental or genetic factors affecting plant growth and development. As stated by Norton (17) in his experiments on beans, the greatest effect of environment seems to be upon such characters as length of the plant axis and probably the twining habit to some extent. These characters and many other such as increased yield and canning quality are related to the genetic make-up of the plant as genes on the chromosome exert their action upon physiological and developmental processes through a controlling effect on the synthesis of enzymes.

A. Environmental Studies

Environment, by definition, is the "aggregate of all the external conditions and influences affecting the life and development of an organism." Among environmental factors widely investigated, temperature and radiant energy have received considerable attention. Although it is not intended to present here a comprehensive review of literature on responses of crops to temperature and to light intensity, quality and duration, a brief discussion of some recent investigations on the subject might be of some interest.

The effects of temperature, photoperiod and age upon growth are reported by Viglierchio and Went (25) who found that, in general, bean plants showed greater stem growth at the higher temperatures. During the early stages the rate of stem elongation is a function of night temperature, increasing with the higher nycto temperatures. They also noticed in their experiment that plants under long day condition grew faster than those under short days. Their conclusions suggest that production of photosynthates may have been limiting growth in the short day regime.

Highkin (8) grew Pea plants (Pisum satiuum) under conditions of constant temperature and artificial light or in controlled-temperature greenhouses in which there was a diurnally fluctuating temperature. The results of these experiments demonstrated that the phenotype of the plant is the summation of its genetic heritage together with the summation of the parent and present environment, and that any environmental effect is inversely proportional to the number of generations by which that environment is removed from the generations under consideration.

As reported by de Zeeuw and Leopold (27), exposure of etiolated pea stem sections to ultraviolet light prior to auxin treatment inhibits the normal growth response preventing auxin action inside the plants.

With the use of a series of wave bands of radiant energy in the blue, red and far red, and wet weight of leaves, inhibition of hypocotyl length and stimulation of epicotyl length were found by Klein et al. (10) to be approximately proportional to the log of the incident energy, with maximum effectiveness in the red at 630.700 millimicrons ($m\mu$), which was markedly more effective than the far red. They also noticed that the increase in relative anthocyanin concentration in the bean hypocotyl similarly was about proportional to the log of irradiance, with the red more effective than the blue and markedly more so than the far red. As suggested in their conclusions, the increased synthesis is coupled in some manner with growth photoreactions. However, Tisdale and Nelson (23), discussing the effect of light quality on plant growth, remark that the results of studies that have been made in this respect on the whole indicate that the full spectrum of sunlight is generally most satisfactory for plant growth.

B. Genetic Studies

Inheritance in beans has been investigated for a long time and in a great extent but still there are many divergent results that call for further studies to provide more reliable bases for greater accuracy in the interpretation of observations. Summary of the literature on *Phaseolus vulgaris* and breeding has been presented by Ten Doorkaat-Koolman (11), Fruwirth (11), and Matsuura (11), cited by Kooiman (11) and Wade (26).

When classified as to type of vine, beans are referred in the literature as "bushy" or "viny." A bush bean, as defined by Wade (26), is a type in which the inflorescence is at the tip of the plant; when it appears the plant stops growing. In a viny or pole bean, on the other hand, the flowers are along the stem, which continues to grow indefinitely; its ultimate length depends on environmental conditions.

To Emerson (4), pole and bush beans also differ in height in consequence of the difference in habit of growth. Bush beans, he comments, have a short axis in part because of a small number of internodes and in part also because of a small mean internode length, the latter being due to termination of the plant axis in the period of growth rate acceleration. Pole and bush types, then, are characterized by difference in height, only in so far as height is dependent upon determinate and indeterminate habits of growth.

Mendel (15) crossing tall and dwarf forms of beans found that tallness is dominant to dwarfness and that F_2 segregated in a 3:1 ratio of tall to dwarf. With similar crosses the same results were obtained by McRostie, Tjebbes and Kooiman, and Doornkaat-Koolman, and quoted by Wade (26).

Dealing with the determinate versus the indeterminate habit of growth as a separate character instead of limiting his investigation to the general character of tall versus short habit of growth, Emerson (4) shed more light on the subject. He showed that there are three factors involved in plant height, namely:

1. determinate versus indeterminate habit of growth;

2. number of internodes, which in pole beans nevertheless depends largely on environmental conditions;

3. internode length, which is greater in the hypocotyl than the epicotyl which in turn is longer than the second internode.

Indeterminate habit of growth is found to be fully dominant to determinate habit. Sharp segregation occurs in F_2 resulting in a 3:1 ratio of indeterminate to determinate habit. This recessive trait is constant in F_3 while some indeterminate F_2 plants breed true in F_3 and others segregate again into pole and bush beans. Difference in habit of growth, therefore, appears to be a unit character. This opinion is also held by Sax (21) and Makarem (14) reporting Hilpert's conclusion on the subject. Emerson also observed that while number of internodes is directly, and internode length indirectly related to habit of growth, these characters are in a way distinct from it. There exist distinct types of bush beans and distinct types of pole beans with respect to both number of internodes and internode length. Crossing varieties of bush beans with different internode lengths seems to result in an intermediate condition in F_1, and F_2, and a wide range of variation in the latter. This was interpreted by Emerson in accordance with the multiple factor hypothesis which postulates that hereditary quantitative differences are due to two or more non-dominant, independently inherited factors.

Norton (17) asserts that the habit of all the varieties of beans can be accounted for easily with only three characters: A, L and T. "A" designates the presence of axial inflorescence permitting an indefinite growth of the main axis and main branches; its recessive counterpart "a" stands for a terminal inflorescence causing definite growth; "L" the length of axis, an important factor controlling plant habit and probably governed by a series of two or more factors which behave after the fashion of Emerson's hypothesis for the inheritance of quantitative characters; "T" the climbing habit, is due to a factor for circumnutation. The cause of the various degrees of the climbing habit has not been determined with any degree of certainty, while the contorted stems of erect bush forms are thought to be probably caused by T. Furthermore it is assumed that A, L and T may be present in any possible combination giving rise to the various habit types of beans.

Later Lamprecht (12) proposed "Fin" to replace the symbol A used by Norton. He also replaced T by Tor to describe the twining habit. Analyzing results obtained from a cross between a tall type of Phaseolus vulgaris and a slender type, which differs from the latter by its excessively long internodes, Lamprecht reached the conclusion that infinite "Fin," long "L," climbing axis "Tor" are respectively dominant to finite growth of the axis "fin," short internodes "l" and not climbing axis "tor."

He also reported results of crosses made by de Haan (1927) and Rasmusson (1927) between certain families of dwarf peas. Simultaneously two ratios of 15:1 and 3:1 dwarfs to cryptodwarfs were computed in this case. Two symbols "Cry_1" and "Cry_2" (Rasmusson) or "La" and "Lb" (de Haan) were assigned to be responsible for the dwarf-cryptodwarf character. Besides, a cross of dwarf x dwarf types of *Phaseolus vulgaris* made by Lamprecht (12) yielded a ratio of 15:1 dwarfs to slender. Such a result caused him to infer that the slender type is caused by recessivity in two genes for the length of the internodes "Cry" and "La;" each in dominant condition preventing the length growth of the internodes. Moreover, he pointed out that for other characters, such as pods, there also seems to exist in *Phaseolus vulgaris*,as it is in *Pisum sativum*, a number of polymeric genes which as "Cry" and "La" may be taken as an evidence that *Phaseolus vulgaris* has arisen from a tetraploid ancestor by loss of one or more chromosomes.

The foregoing discussion substantiates the statement that genetics of beans is quite complicated and that penetrating investigations are required to find an answer to many intriguing and important questions.

C. Developmental Studies

Whether due to environmental or to genetic influences, phenotypic dissemblances between or within species may evolve from striking differences in the anatomical features of the living organisms under comparison. These differences may be related to cell division, to cell expansion, or to both. Ecological factors such as light and temperature, and certain natural or synthetic substances such as gibberellin and 2-4,D are known to affect plant growth and development

A very abundant literature is available on the study of cell responses to various treatments with light and growth hormones. Without any attempt to cover this broad material in its entirety, a few reports of recent investigations on the subject might be worthy of being cited here.

Humphries and Wheeler (9) working with Kinetin, Gibberellic acid, and light on expansion and cell division in leaf disks of dwarf beans (Phaseolus vulgaris), found that Kinetin increased leaf expansion in the dark wholly by increasing cell size. This effect was eliminated by light. Gibberellic acid also increased cell size and cell division in the dark but not in the light. Light similarly increased cell division and cell size, and although it eliminated the effect of gibberellin on cell division, it enhanced the action of that hormone on cell expansion. The authors brought no clear evidence that light acts by influencing concentration of Kinetin or gibberellin in the leaf but assumed that both substances depress cell division in presence of light.

To Feutcht and Watson (6), application of 20 milligrams of gibberellin on seedlings of Phaseolus vulgaris not only appeared to increase the length of the internodes of the seedlings but seemed also to augment the number and length of cells. In spite of the substantial increase in cell numbers, cell elongation was assumed to be primarily responsible for the increase in internode length.

Conclusions presented later by Greulach and Haesloop (7) on a similar experiment disagree in some extent with Feutcht's assumptions. Growth promotion by Gibberellic acid, as viewed by Greulach and Haesloop, involved only cell division and not cell elongation, and in the pith the growth hormone may have also influenced the plane of cell division. These authors even suggested that earlier conclusions that Gibberellic acid growth promotion is principally due to cell enlargement must be revised.

SECTION I

ENVIRONMENTAL STUDIES

Experiment I

Materials and Methods

Among the commercially grown variety of Navy pea beans, 211-V is conspicuous under certain conditions by its very long hypocotyl section, which is very short in Sanilac on a comparative basis. To study the extent in which certain environmental conditions may affect this agronomic trait the following experiment was set up.

In the fall of 1959, foundation seeds of Sanilac and 211-V were sown in the Plant Science greenhouses of Michigan State University, at East Lansing, Michigan. Eight flats filled with steamed sand were planted with 40 seeds of each variety in five rows of eight seeds each. Rows were four inches apart and with a distance of two inches between seeds in the row. The sand in the flats was leveled with a specially built wooden device and great care was provided to place seeds practically at a uniform depth of one inch to insure identical bedding conditions for uniform germination. After each watering and application of liquid fertilizer, the sand in the flats was maintained at the original level by adding small quantities of wet sand to the flat and working the surface by hand.

Four reciprocal treatments were applied to the materials, namely:

1. Long day--high light intensity.

2. Long day--reduced light intensity,

3. Short day--high light intensity.

4. Short day--reduced light intensity,

so that there were two groups of day length split into two light intensities. Each treatment comprised a set of two flats with the variety arranged in semi-alternate fashion between consecutive flats within a day-group. Varietal arrangement was reversed between groups.

Long day refers to a lighting period of 15 hours and short day to a 9 hour period. Two parallelepipedic wooden frames covered with dark brown and perforated cloth on all but one face provided shading to material under reduced light treatment. Similar boxes wrapped in black and solid cloth were used on the materials placed under short day conditions, after the 9 hour period of exposure to light was attained. Materials under long day, high light treatment were kept unshaded throughout the experiment. Day lighting period in the fall for Michigan more or less lasts 9 hours, the sun rising at about eight o'clock in the morning and setting at about five o'clock in the evening. To extend light exposure to 15 consecutive hours, eight 1250 foot candle bulbs were mounted on a circuit switched to an automatic clock timer that turned on the lights at 5 p.m. and cut them off at 11 p.m. Lights were kept on during dark days to supplement the normal day light period of 9 hours.

For the sake of convenience, 22 days after emergence of the seedlings, the hypocotyl section was considered to have reached full development. The plants were then pulled up and measurements of their hypocotyl taken in half centimeters with a graduated ruler. Abnormal seedlings were discarded, and to deal with a same number of individuals in each treatment, a random sample of 25 seedlings of each variety in each flat was selected and mean hypocotyl lengths of each variety in each treatment calculated on a basis of 50 measurements.

Experimental Results and Discussion

Plate II and Plate III illustrate typical behaviors of both Sanilac and 211-V under full light and reduced light conditions for a 15 hour period of illumination corresponding to the long day treatments. Actual measurements and computed average hypocotyl lengths in this first experiment are recorded in Table 1.

From a mean length of 5.00 cms under long day and 5.14 cms under short day treatments with high light intensity applied, the hypocotyl section of Sanilac reached an average height of 7.76 cms under long day and of 8.17 cms under short day treatments when light intensity is reduced by shading.

Exposed to full light condition, 211-V decreases from an average hypocotyl length of 11.22 cms in the long day to a mean length value of 9.52 cms in the short day regimes. Under reduced light treatment this variety still decreases with day length, shifting from an average hypocotyl length of 12.87 cms

TABLE 1. Experiment I. Hypocotly length of Sanilac and 211-V for treatments.

	Long day--High light									
	Sanilac					211-V				
Flat 1	6.0	5.5	5.0	6.0	5.5	11.5	11.0	10.5	12.5	10.5
	5.0	4.0	5.0	4.5	4.0	12.5	10.5	10.5	11.5	10.5
	6.0	5.0	4.5	5.0	5.0	11.0	12.0	12.5	10.5	11.0
	5.5	5.5	5.0	5.0	5.0	12.0	12.5	10.5	11.0	10.5
	6.0	5.0	4.5	5.5	4.5	11.0	15.5	11.5	10.5	11.0
2	4.5	5.5	5.5	4.5	6.0	11.0	10.5	11.5	12.0	11.0
	5.0	5.0	5.0	4.0	4.5	11.0	10.5	12.0	11.0	11.5
	6.0	5.0	5.0	4.5	5.0	10.5	10.5	10.5	10.5	10.5
	6.0	4.5	4.0	5.0	5.0	12.0	11.5	11.5	11.0	10.5
	5.0	4.0	4.5	4.5	5.0	10.5	10.5	11.5	10.5	12.5
Means			5.00					11.22		

TABLE 1 Continued

	Long day--Reduced light									
	Sanilac					211-V				
Flat 1	10.0	8.0	8.0	8.5	7.0	12.0	12.5	14.0	13.0	12.0
	10.0	6.5	8.0	7.5	7.0	14.5	13.5	12.5	11.5	11.5
	10.0	9.0	8.0	7.0	8.0	15.0	12.5	14.0	11.5	13.5
	7.5	8.0	8.0	9.0	8.5	14.0	14.0	13.5	12.5	11.0
	9.0	8.0	7.5	7.5	10.0	14.5	13.0	11.5	12.0	12.5
2	10.0	8,0	8.0	7.0	7.0	12.5	11.5	12.0	13.5	12.5
	9.0	9.5	8.5	7.5	8.0	12.0	14.5	11.5	13.5	12.5
	8.5	8.5	8.5	7.0	7.0	13.5	11.5	12.5	11.5	14.0
	8.5	8.5	7.5	8.0	8.0	13.5	13.5	13.0	12.0	14.0
	10.0	7.0	7.0	8.5	8.0	14.5	14.5	13.5	12.0	12.0
Mean			8.17					12.87		

TABLE 1 Continued

	Short day--High light									
	Sanilac					211-V				
Flat 1	5.5	5.0	5.0	5.0	6.0	11.5	10.0	9.5	10.0	9.5
	5.0	5.5	5.0	5.5	5.5	10.0	9.0	10.0	10.5	9.5
	5.0	4.5	5.5	5.0	6.0	11.0	11.0	9.0	10.0	9.0
	5.0	6.0	4.5	5.0	4.0	9.0	11.0	9.5	9.5	9.5
	6.0	6.0	4.5	5.5	5.0	10.0	9.5	9.5	11.0	9.0
2	6.0	4.5	5.5	4.5	5.0	9.5	9.0	8.5	8.5	8.5
	5.5	6.0	5.0	5.0	5.0	9.5	9.5	9.0	8.5	9.5
	6.0	5.0	5.0	4.5	5.0	9.5	9.0	9.0	9.0	9.0
	5.0	4.5	4.5	6.0	5.0	9.5	9.0	8.5	9.5	9.0
	5.5	5.0	5.0	4.5	4.0	9.5	8.5	9.5	11.0	9.5
Means			5.14					9.52		

TABLE 1 Continued

	Short day--Reduced light									
	Sanilac					211-V				
Flat 1	7.5	7.5	8.0	7.5	7.0	14.5	13.0	12.5	12.5	12.5
	9.0	8.5	7.0	7.0	8.5	15.0	14.0	12.5	12.5	12.0
	9.0	8.5	9.0	8.0	7.0	13.5	12.0	12.0	12.0	12.5
	7.5	8.0	8.5	7.0	8.0	14.0	12.5	12.5	14.5	12.5
	9.0	8.0	7.0	7.0	7.0	12.0	12.5	12.0	12.0	13.5
2	9.0	8.0	7.5	7.5	8.0	14.0	12.0	11.5	13.5	14.5
	9.0	7.5	7.0	8.0	7.5	12.5	11.5	11.5	14.0	11.5
	8.5	8.0	7.0	7.5	7.0	15.0	11.5	11.5	11.5	13.0
	7.0	8.0	8.0	7.0	7.5	12.5	12.5	13.0	12.0	12.5
	9.0	7.0	7.0	7.0	7.5	11.5	11.5	13.5	11.5	11.5
Means			7.76					12.64		

in long day condition to a corresponding value of 12.64 cms under short day treatment.

Analyzing these observations, reduced light intensity seems to have a greater effect than full light in increasing the hypocotyl section of either variety. Actually as indicated by analyses of variance set up in Table 2 and Table 3, differences due to light intensity are highly significant in both cases. Variations of Sanilac and 211-V in relation to light treatment are plotted in Figure 2.

These results though striking are not surprising and were rather normally expected as it is generally observed and reported that leaf size and stem elongation of many species are at a maximum at low light intensities. Most plants when shaded are taller and thinner than when exposed to full light. Etiolation of seedlings in crowded stand is a very common phenomenon.

Considering effect of photoperiodism on Sanilac and 211-V with respect to their hypocotyl elongation, it can be easily seen in Figure 3 that while 211-V constantly decreases when passing from long day to short day conditions, Sanilac on the other hand, displays greater hypocotyl lengths in short day than in long day when submitted to high light intensities but decreases from long day to short day when under reduced light intensity. As it is revealed

TABLE 2. Analysis of variance of hypocotyl elongation of Sanilac under four day and light treatments.

Source of Variation		D.F.	S. Sq.	M. Sq.	F.
Total		199	522.69		
	Day	1	.91	.91	1.82
Treatments	Light	1	419.05	419.05	838.10**
	D x L	1	3.78	3.78	7.56**
Error		196	98.94	.50	

**Significant at the 1% level.

TABLE 3. Analysis of variance of hypocotyl elongation of 211-V under four day and light treatments.

Source of Variation		D.F.	S. Sq.	M. Sq.	F.
Total		199	531.47		
	Day	1	46.56	46.56	52.90**
Treatments	Light	1	284.41	284.41	323.19**
	D x L	1	27.01	27.01	30.69**
Error		196	173.49	.88	

**Significant at the 1% level.

Hypocotyl Elongation of Sanilac and 211-V

Figure 2
Response to light intensity

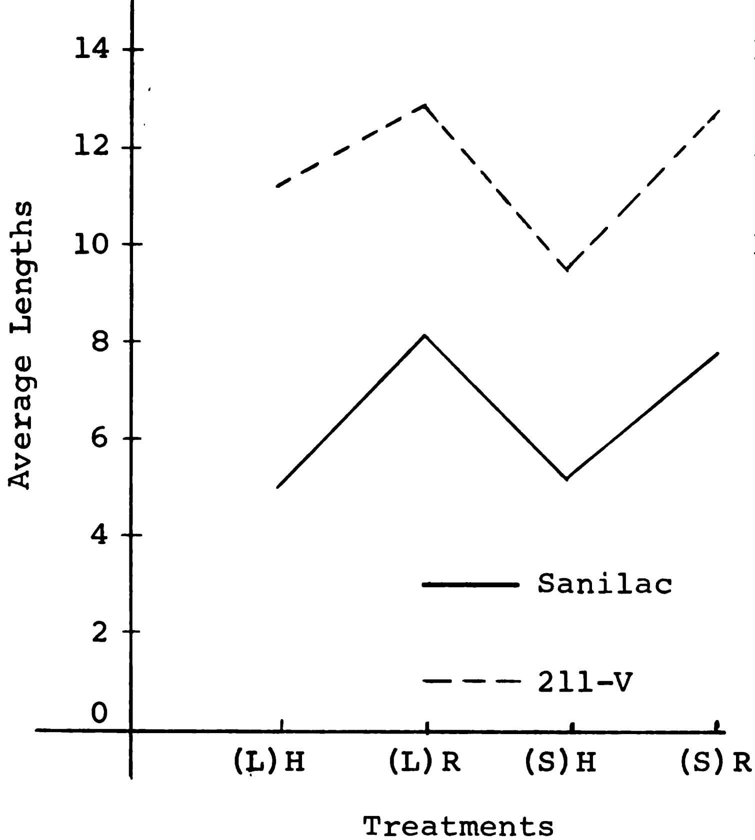


Figure 3
Response to day length

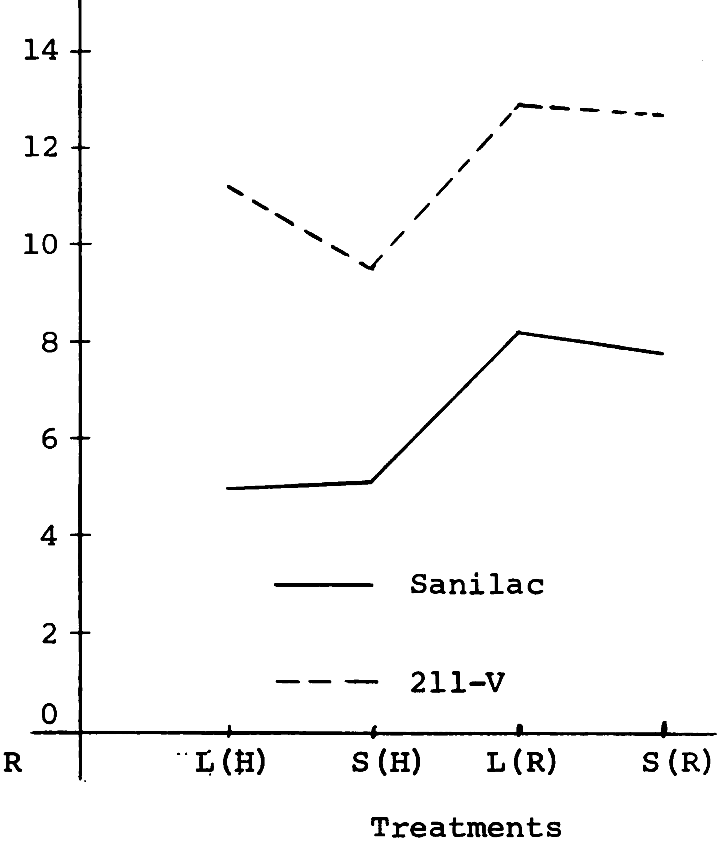
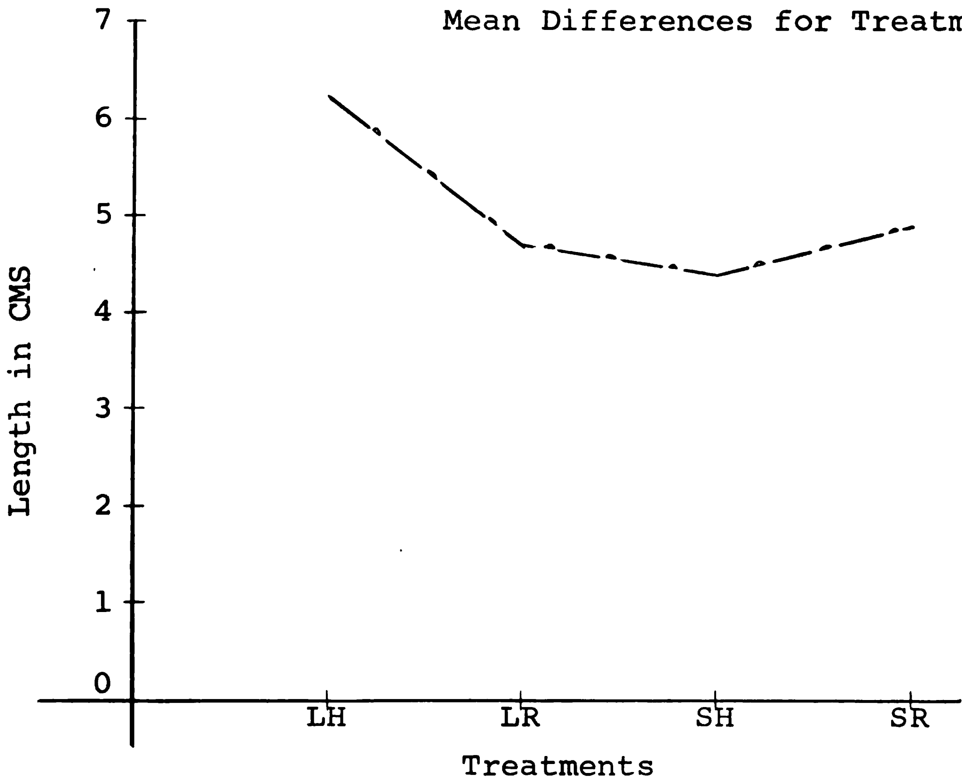


Figure 4
Mean Differences for Treatments



by t-tests in Table 4 and Table 5, there is no indication that day length affects hypocotyl growth rate of Sanilac while 211-V fails to manifest consistent responses in this sense. The latter strain of Navy bean appears to be indifferent to duration of exposure to light under shading but shows a significantly higher hypocotyl average length in long day than in short day when full light conditions are prevailing.

A curve plotting differences between varieties for each type of day-light treatment is made available in Figure 4. An analysis of variance for the whole experiment clearly indicates in Table 6 that there are highly significant differences between mean hypocotyl lengths of Sanilac and 211-V for treatments and using the Student Range Method to test differences among means, it has been found that any mean value of 211-V is always significantly superior to the corresponding mean value of Sanilac for any given treatment.

Summary and Conclusions

By testing variations in hypocotyl lengths of Sanilac and 211-V for significant differences between their relative mean values under the various treatments applied, it has been deducted that both varieties develop a higher hypocotyl section with reduced light intensity than under full light condition.

The bush variety shows little change with respect to length of day whereas the vine type behaves rather inconsistently,

TABLE 4. A t-test of effect of day length treatment upon Sanilac.

S_L	=	$\sqrt{\dfrac{4647.75 - 4336.22}{99}}$	=	$\sqrt{3.14}$	
S_S	=	$\sqrt{\dfrac{4370.50 - 4160.25}{99}}$	=	$\sqrt{2.12}$	
$S_{\bar{L}}$	=	$\dfrac{\sqrt{3.14}}{\sqrt{100}}$	=	$\dfrac{\sqrt{3.14}}{10}$	
$S_{\bar{S}}$	=	$\dfrac{\sqrt{2.12}}{\sqrt{100}}$	=	$\dfrac{\sqrt{2.12}}{10}$	
$S_{(\bar{L} - \bar{S})}$	=	$\sqrt{\dfrac{3.14 + 2.12}{100}}$	=	.23	
t	=	$\dfrac{6.58 - 6.45}{.23}$	=	.56	N.S. (3)

SL = Standard deviation for long day.
SS = Standard deviation for short day.
N.S. = Not significant.

TABLE 5. A t-test of effect day length treatment on 211-V.

S_L	=	$\sqrt{\dfrac{14672.25 - 14508.20}{99}}$	=	$\sqrt{1.66}$
S_S	=	$\sqrt{\dfrac{12597.50 - 12276.64}{99}}$	=	$\sqrt{3.24}$
$S_{\bar{L}}$	=	$\dfrac{\sqrt{1.66}}{\sqrt{100}}$	=	$\dfrac{\sqrt{1.66}}{10}$
$S_{\bar{S}}$	=	$\dfrac{\sqrt{3.24}}{\sqrt{100}}$	=	$\dfrac{\sqrt{3.24}}{10}$
$S_{(\bar{L} - \bar{S})}$	=	$\sqrt{\dfrac{1.66 + 3.24}{100}}$	=	.22
t	=	$\dfrac{12.04 - 11.08}{.22}$	=	4.36**

SL = Standard deviation for long day.
SS = Standard deviation for short day
** = Significant at the 1% level.

TABLE 6. General analysis of variance of Sanilac and 211-V under different treatments of day length and light intensity.

Source of Variation		D.F.	S.Sq.	M.Sq	F.
Total		399	3599.36		
Treatments	Day length (D)	1	30.25	30.25	21.92**(1)
	Light intensity (L)	1	696.96	696.96	505.04**
	D x L	1	5.29	5.29	3.83
Varieties (V)		1	2545.20	2545.20	1844.35**
D x V		1	17.22	17.22	12.48**
L x V		1	6.50	6.50	4.71
D x L x V		1	25.51	25.51	18.48**
Error within		8	11.03	1.38	
Other errors		384	261.40		

ANNEX TO TABLE 6. Student range test for hypocotyl means of Sanilac and 211-V.

S.D. of a treatment average: $S_{\bar{T}} = \sqrt{\frac{1.38}{2}} = .83$

Significant studentized ranges for d. = 8 (P/N_2)

[2] 4.74 x .83 = 3.93

Treatments	Variety	Means	Difference
Long day high light	211-V - Sanilac	11.22-5.00	6.22**
Long day reduced light	211-V - Sanilac	12.87-8.17	4.70**
Short day high light	211-V - Sanilac	9.52-5.14	4.38**
Short day reduced light	211-V - Sanilac	12.64-7.76	4.88**(1)

(1)**Significant at the 1% level.

revealing a significantly greater hypocotyl mean height during long day than during short day when maintained under full light exposure but standing practically unaffected by photoperiodicity when shaded.

Comparing hypocotyl elongation of these two varieties for response to combined effect of day length and light intensity, greater difference between their mean hypocotyl length has been noticed to occur under long day high light intensity than under any one of the three remaining combinations. Nevertheless, throughout the experiment hypocotyl mean sections of 211-V have never failed to be significantly longer than that of Sanilac irrespectively of the treatment considered. This observation may suggest that besides environmental influences and more than them intrinsic factors are responsible for this tangible and consistent difference in hypocotyl growth rate of these two varieties.

A

B

FIGURE 5. Plate II. Young seedlings of Sanilac under long day full light intensity treatment.

Young seedlings of 211-V under long day full light intensity treatment.

A

B

FIGURE 6. Plate III. Young seedlings of Sanilac under long day reduced light intensity treatment.

Young seedlings of 211-V under long day reduced light intensity treatment.

SECTION II

GENETIC STUDIES, EXPERIMENT II, PART I

Materials and Methods

In 1959 crosses between Sanilac and 211-V were made and the seeds obtained from such crosses preserved for genetic studies. In March, 1960, an experiment to compare growth habit of F_1 seedlings to that of the parents was undertaken. Eight pots filled with steamed sand and organic matter received each: four F_1 seeds and two seeds of either parental varieties under no special environmental condition in the greenhouse room. Liquid fertilizer was supplied twice to the pots. When seedlings had achieved a fairly advanced stage of development their hypocotyl section was measured in a single operation with a ruler graduated in half-centimeters. Then each plant was supplied with an iron wire to allow twining and keep them from entanglement. Care was provided in handling materials to avoid damage as further crosses and harvest were planned. Beans are somewhat difficult to hand-pollinate since the curled and brittle style of the flowers is easily broken during the process of opening the keel. Wade (26) states that the time required to make crosses has prevented genetic studies in Phaseolus vulgaris involving back-crosses. Nevertheless, remarked he, keeping atmosphere near saturation point a few days after artificial pollination has been effected may yield fairly successful crosses.

To produce F_1 seeds, Sanilac was used as the maternal parent and 211-V as pollen donor. Back crosses to Sanilac in the present studies are termed BC_s and to 211-V, BC_v.

Crosses were made by selecting flowers of the female parent before eclosion to minimize possibility of selfing. Emasculation was effected by pinching the base of the flower and forcing the reproductory organs to protrude and, using a pair of fine tweezers, carefully driving out the anthers. Stamens, together with the pistil of the male parent, were picked up with tweezers and rubbed onto the stigma of the emasculated flower. Only flowers with mature viable pollen were chosen for fertilization. Pollination was carried out in the early morning with a high moisture level in the atmosphere of the greenhouse room. Tags were placed around the stem of the pollinated flower to indicate the parents involved in the cross and the date of the operation. The female parent was always mentioned first on the tag. Hybrids and selfed seeds harvested from this experiment were saved for further studies.

Experimental Results

Measurements taken on the seedlings and average hypocotyl lengths of each type of plant in the experiment are reported in Table 7. Three seedlings in the F_1 group upon development

TABLE 7. Experiment II (a), Genetic Study.
Hypocotyl lengths of Sanilac, 211-V, and F_1.

Entries	Sanilac	F_1	211-V
1*	10.0	10.0	14.0
	10.0	8.0	14.0
		8.5	
		8.5	
2	5.5	8.5	14.5
	6.0	9.0	14.0
		10.0	
3	6.0	8.0	14.0
	7.0	9.5	13.5
		7.5	
		8.5	
4*	10.5	10.5	13.5
	9.5	8.0	13.5
		10.0	
		10.5	
5*	8.5	10.5	14.0
	11.5	10.5	14.5
		10.5	
		11.5	
6	7.5	9.0	14.0
	7.5	8.0	14.5
		8.5	
		7.5	
7	6.0		14.0
	5.0	9.0	14.0
		9.5	
8	7.5	8.0	14.5
	6.5	9.0	14.0
		8.5	
		7.5	
Total	124.5	262.5	224.5
Means	7.78	9.05	14.03

*Plots placed very close to hot pipes in the greenhouse.

turned out to be accidental selfed plants and were disregarded in computing average values of either group. Averages obtained were 9.05 cms for F_1, 7.78 cms for Sanilac, and 14.03 cms for 211-V.

All the true F_1 plants showed the viny character of 211-V but indicated a mean hypocotyl height closer to that of Sanilac than to that of 211-V. Crosses and back crosses of F_1 to either parent were satisfactorily successful and yield obtained from each group of plants was abundant and supplied enough material for the next experiments.

An analysis of variance carried out in Table 8, reveals that there is highly significant difference among hypocotyl mean lengths of the three classes in this experiment. Using the Student Range Method for testing significance among average measurements it has been obtained, as shown in the Annex Table to Table 8, that while hypocotyl mean length of 211-V is significantly greater than that of either Sanilac or F_1, there is no significant difference between the bush parents and F_1 with regard to their average hypocotyl growth rate.

Plants growing in pots placed near the heating pipes on the benches in the greenhouse room have been found to elongate more than those grown in pots located farther from the heat source.

TABLE 8. Analysis of Variance for hypocotyl elongation of Sanilac, F_1, 211-V.

Source of Variation	D.F.	SS	M.sq	F
Total	60	460.25		
Between classes	2	95.38	47.69	7.58**
Error	58	364.87	6.29	

**Significant at the 1% level.

ANNEX TO TABLE 8. Student Range Test for hypocotyl means of Sanilac, F_1, 211-V.

$$N_O = \frac{1}{2}\left(61 - \frac{1363}{61}\right) = 19.41$$

$$\text{S.D. of a class average} = \sqrt{\frac{6.29}{19.41}} = .57$$

Means of classes	1	:	2	:	3
	211-V		F_1		Sanilac
	14.03		9.05		7.78

Significant Studentized Ranges at the 1% level with 58. d.f.

[2] 3.76 x .57 = 2.14

[3] 3.92 x .57 = 2.23

Comparisons	Respective means	Differences
F_1 - Sanilac	9.05 - 7.78	1.27 N.S.
211-V - F_1	14.03 - 9.05	4.98**
211-V - Sanilac	14.03 - 7.78	6.25**

N.S. Not significant.
**Significant at the 1% level.

Discussion of Results

Inheritance of the character "internode length" and specially hypocotyl length in common beans is less strongly established than that of the agronomic character "determinate versus indeterminate" type of growth, another component of plant height. Experimental papers on the subject, such as those published by Emerson (4), Norton (17), and others have reported that F_1 plants of crosses between parents of different internode lengths are of an intermediate condition. Emerson has adopted the multiple factor hypothesis to explain his findings. However, there is no complete agreement as to the number of genes responsible for the transmission of the trait. Lamprecht (12), backing Rasmusson and de Haan's conclusions, stated that long internode in beans (Phaseolus vulgaris) is due to recessivity in two genes for the length of the internode, each in dominant condition preventing the length growth of the internode. Rasmusson and de Haan (1927) have respectively suggested the symbols Cry_1, Cry_2, and La, Lb to designate these factors, which later were represented as Cry La by Lamprecht (12). None of the reports consulted in the review of literature for the preparation of the present paper offers special mention to the hypocotyl section of the plant, which is not considered as a true internode by many plant anatomists, as pointed out by Esau (5).

As it happens that there is no significant difference between Sanilac and F_1 in their respective hypocotyl mean heights while both are significantly shorter than 211-V, and taking into account the slight but appreciable advantage of F_1 to Sanilac in average hypocotyl length growth, it could be assumed that short hypocotyl is partially dominant to the long hypocotyl trait. This slight difference between mean lengths of Sanilac and F_1 could also be due to hybrid vigor in the latter. So far the hypothesis of non-dominance, which would result in an intermediate condition in the first generation of plants may be disregarded. At this stage of the study, however, it is too early to assign any specific number of genes as responsible for the transmission of this character. Subsequent generations of selfing of the hybrids and appropriate back crossings may lead to more information and more accuracy in this respect.

Summary

Crosses between Sanilac, a bush bean with short hypocotyl and 211-V, a viny type with long hypocotyl, have resulted in viny F_1 plants which affected a mean hypocotyl length very close to that of the short internode parent. This and other pertinent considerations call for culturing subsequent generations of selfed hybrid and back crossed plants to test the possibility

of a partial or complete dominance of short hypocotyl to long hypocotyl section in Phaseolus vulgaris. No evidence is found to sustain a non-dominance hypothesis. At this stage of the study no specific number of factors can be assigned as responsible for the inheritance of hypocotyl elongation in common beans.

EXPERIMENT II, PART II

Materials and Methods

In late spring, 1960, seeds of Sanilac, 211-V, F_1, BC_s, and BC_v were sown in ten flats similar to those used in the experiment on environmental studies the preceding fall. Each flat was furrowed into eight rows, containing eight seeds each. There were 15 rows of F_2, 10 of each parent, and five of F_1, BC_s, and BC_v, respectively. The parent variety stood for check materials. Even level of sand in the flats was realized and same depth of planting observed to insure uniform germination. When satisfactorily developed, all seedlings were measured the same day for hypocotyl elongation. Afterwards F_2 and back cross seedlings were retransplanted in large pots filled with sand and organic matter so that distinction could be made among segregates as to the bushy or viny types of growth.

Experimental Results

Measurements were taken for 80 seedlings of Sanilac, 80 of 211-V, 26 of F_1, 39 of back cross of F_1 to Sanilac, 40 of back cross of F_1 to 211-V, and 117 of F_2 seedlings. Some bushy plants were found in the F_1 lot. Possibly they might have arisen from selfed seeds during the process of hybridization and consequently were disregarded as pertaining to the F_1 group. Experimental results are shown in Table 9 through Table 14. For each group of plants hypocotyl lengths are distributed in classes of one cm of interval and their mean values computed on the basis of actual measurements. The following averages were calculated in accordance with this procedure: 5.66 cms for Sanilac, 12.91 for 211-V, 7.03 for F_1, 6.14 for BC_s, 11.61 for BC_v, 8.58 for F_2. Standard deviations were very small in the case of Sanilac and F_1, being smaller than 1 cm. It was relatively high for BC_v, reaching nearly 3 cms.

Discussion of Results

A consideration of the mean hypocotyl lengths together with the appropriate standard deviations for Sanilac and 211-V in relation to the mean elongation growth of the hypocotyl of the F_1 progeny, may bring out some indication regarding the mode of inheritance involved in the transmission of this trait.

By its mean value, the F_1 generation is closer to the

TABLE 9. Experiment II (b). Hypocotyl Elongation of Sanilac.

1.	4.0	6.0	6.5	6.0	6.0	6.0	4.5	4.0
2.	4.5	5.5	6.5	7.5	5.0	5.0	4.0	4.0
3.	5.0	5.0	6.5	7.5	5.0	6.0	5.0	5.0
4.	4.0	5.5	7.5	7.0	6.0	6.5	6.0	7.0
5.	4.5	5.5	6.5	6.5	5.5	5.5	6.5	5.0
6.	4.0	5.5	6.0	6.5	5.5	4.0	5.5	5.0
7.	5.0	7.0	6.0	6.5	5.5	6.0	6.0	5.5
8.	5.0	6.0	6.0	5.5	7.0	4.5	7.0	5.5
9.	7.0	5.0	6.0	6.5	6.0	6.0	6.0	4.0
10.	6.5	4.5	6.5	7.5	4.0	5.0	6.5	5.0

Mean $\overline{X}$ = 5.66

S.D. = .96385

Distribution

Class	Frequency
3.5 - 4.4	9
4.5 - 5.4	19
5.5 - 6.4	29
6.5 - 7.4	19
7.5 - 8.4	4
Total	80

TABLE 10. Experiment II (b). Hypocotyl Elongation of 211-V.

1.	11.5	13.0	11.0	14.0	15.5	14.0	14.0	13.5
2.	11.0	12.0	14.0	12.0	12.0	10.5	14.0	14.0
3.	10.5	12.5	12.0	13.5	10.5	13.0	14.0	13.5
4.	11.5	13.0	15.0	15.0	10.5	14.5	14.5	11.0
5.	11.0	12.0	13.0	13.5	12.0	14.0	13.0	11.0
6.	10.5	13.0	14.0	15.0	11.0	14.0	11.0	15.5
7.	11.0	15.5	14.0	15.0	15.5	13.5	13.0	11.5
8.	12.5	13.0	14.0	15.0	11.0	14.0	12.5	12.0
9.	14.0	14.5	11.0	12.5	14.5	11.5	11.5	10.5
10.	13.0	14.5	12.0	15.0	14.0	13.5	11.0	13.0

Mean $\overline{X}$ = 12.91

S.D. = 1.4967

Distribution

Class	Frequency
10.5 - 11.4	17
11.5 - 12.4	13
12.5 - 13.4	14
13.5 - 14.4	21
14.5 - 15.4	11
15.5 - 16.4	4
Total	80

TABLE 11. Experiment II (b). Hypocotyl Elongation of F_1.

			F_1			
1.	8.5	8.0	6.5	7.5	6.5	7.0
2.	6.0	8.0	2.0	5.5	6.5	
3.	6.0	6.5	8.5	8.5	6.5	
4.	7.5	7.0	7.0	6.5	7.5	
5.	7.5	7.5	5.5	7.0	7.0	

Mean $\overline{X}$ = 7.03

S.D. = .847

Distribution

Class	Frequency
5.5 - 6.4	4
6.5 - 7.4	12
7.5 - 8.4	7
8.5 - 9.4	3
Total	26

TABLE 12. Hypocotyl Elongation of BC_s.

				BC_s				
1.	5.5	7.0	8.0	6.0	6.0	6.0	7.0	6.0
2.	7.0	8.0	6.5	6.0	6.5	4.5	5.5	5.5
3.	5.0	7.0	4.5	4.0	6.0	7.0	6.0	4.5
4.	4.0	6.5	7.0	6.0	4.0	7.0	7.0	5.0
5.	8.5	7.0	6.0	5.0	6.0	8.0	7.5	

Mean $\overline{X}$ = 6.14

S.D. = 1.17

Distribution

Class	Frequency
3.5 - 4.0	3
4.5 - 5.4	6
5.5 - 6.4	13
6.5 - 7.4	12
7.5 - 8.4	4
8.5 - 9.4	1
Total	39

TABLE 13. Experiment II (b). Hypocotyl Elongation of BC_v.

1.	10.0	10.0	14.5	7.0	12.0	13.0	13.5	12.0
2.	11.0	6.5	13.5	12.5	14.5	13.0	14.0	13.5
3.	12.5	11.5	14.0	14.5	13.5	6.0	7.5	11.0
4.	14.0	12.0	7.0	15.0	15.5	11.0	5.5	14.0
5.	13.0	7.0	11.0	14.5	7.0	14.5	14.5	8.0

Mean $\bar{X}$ = 11,61

S.D. = 2.9343

Distribution

Class	Frequency
5.5 - 6.4	2
6.5 - 7.4	5
7.5 - 8.4	2
8.5 - 9.4	0
9.5 -10.4	2
10.5 -11.4	4
11.5 -12.4	4
12.5 -13.4	5
13.5 -14.4	8
14.5 -15.4	7
15.5 -16.4	1
Total	40

Ratio 9:31

TABLE 14. Hypocotyl elongation of F_2.

1.	7.0	7.0	11.0	8.5	5.5	8.0	14.0
2.	6.0	12.5	7.0	11.5	7.5	6.5	7.0
3.	11.0	7.0	7.0	13.0	8.0	12.5	14.0
4.	5.0	7.0	7.0	8.5	10.5	7.0	8.0
5.	7.0	8.5	7.0	7.5	5.5	7.0	13.0
6.	7.5	6.0	7.0	7.0	6.0	7.0	13.0
7.	10.0	13.5	7.0	4.0	12.5	6.0	6.0
8.	6.0	4.0	7.0	12.0	6.5	6.0	8.5
9.	6.0	8.5	10.0	9.0	9.0	12.0	6.5
10.	13.0	4.0	10.0	6.5	6.0	10.0	6.5
11.	5.0	4.5	12.5	12.0	9.0	12.5	5.0
12.	5.0	11.5	13.0	9.5	7.0	6.0	9.0
13.	5.0	5.5	13.0	5.5	7.0	12.5	7.5
14.	6.0	4.0	12.5	6.0	7.0	7.5	7.5
15.	4.0	10.0	4.0	7.0	5.0	5.5	14.0
16.	5.5	10.5	12.0	7.0	6.5	4.0	10.0
17.	10.0	5.0	10.0	5.5	12.0	8.0	12.0
18.	6.0	5.5	4.0	8.0	7.0	5.5	9.0
19.	10.0	10.5	13.0	12.0	10.0	12.5	12.5
20.	8.0	5.0	11.0	5.5	11.5	7.0	13.0
21.	11.0	5.5	8.0	8.0	8.5	6.0	7.0
22.	7.0	7.0	8.0	5.5	7.5	13.5	7.0
23.	7.0	8.0	6.0	6.0	8.0	14.0	10.5
25.	12.0	6.0	12.0	12.5	5.5	6.0	6.0

TABLE 14 Continued

1.	6.5	8.5	9.0	4.0	6.0	9.0	5.5	12.0
2.	9.0	11.5	9.0	6.0	7.0	13.0	5.5	6.5
3.	13.5	7.5	8.5	10.5	6.0	11.0	7.0	7.0
4.	12.5	13.5	9.0	12.5	8.0	8.0	5.5	7.0
5.	6.5	14.0	10.0	6.5	13.5	6.0	9.0	
6.	4.0	10.0	8.0	5.5	10.0	12.0	6.0	
7.	7.0	6.0	14.0	8.0	8.0	10.5	6.5	
8.	9.0	8.5	6.0	8.0	9.0	6.5	6.5	
9.	8.5	13.0	8.5	5.0	5.0	4.5	13.5	
10.	5.5	8.5	8.5	11.0	5.0	6.5	6.0	
11.	12.5	7.0	7.5	8.0	8.5	7.0	6.5	
12.	6.0	13.5	13.5	7.5	5.5	6.5	14.5	
13.	12.0	9.0	12.5	13.5	11.0	6.5	11.0	
14.	11.5	8.0	10.0	12.0	4.5	12.0	7.5	
15.	14.0	11.5	7.0	8.0	5.0	7.5	6.5	
16.	12.0	7.0	11.5	14.0	6.5	4.0	5.5	
17.	15.0	9.0	11.0	8.0	9.0	11.5	12.0	
18.	6.0	7.5	7.0	7.0	7.0	7.0	6.5	
19.	6.0	9.0	4.0	14.5	4.0	4.0	11.0	
20.	13.0	9.5	8.0	11.5	5.0	6.5	7.0	
21.	6.5	6.5	4.0	7.5	7.0	6.0	5.5	
22.	11.0	14.0	7.5	6.0	5.5	7.5	5.5	
23.	5.5	7.0	9.0	7.5	12.5	15.0	8.0	
24.	9.5	6.5	4.0	7.0	6.5	7.0	5.0	
25.	8.5	12.5	5.0	10.5	13.5	14.0	4.5	

TABLE 14 Continued

Distribution	
Class	Frequency
3.5 - 4.4	16
4.5 - 5.4	19
5.5 - 6.4	56
6.5 - 7.4	75
6,5 - 8.4	41
8.5 - 9.4	35
9.5 -10.4	17
10.5 -11.4	18
11.5 -12.4	25
12.5 -13.4	27
13.5 -14.4	20
14.5 -15.4	4
15.5 -16.4	1
	354

Mean $\overline{X}$: 8.58

Ratio: 207:147

short than to the elongate parents. In a normal distribution more than 97 per cent of the area under the curve would be included between Z values of $\mu + 2\sigma$, where z represents standard scores, μ the average value considered, and σ the standard deviation of the population. On the basis of the statistical properties of this distribution the area covered within the range of variation of Sanilac includes the mean length of the F_1 population while this last value would be exterior to the limits of 211-V. Extending these considerations to the F_2 phenotypes, it would be noticed that 207 of the plants in this particular group would fall into the short category, whereas only 147 of them would belong to the elongate type.

On the assumption that a partial dominance of short hypocotyl to long hypocotyl prevails, most of the plants resulting from a back cross of F_1 to the short parent would show an average hypocotyl length lying within the range of the latter. This expectation seems to be actually justified by the distribution of lengths in the BC_s as reported in Table 12.

Nevertheless, determining the number of controlling factors acting upon this trait presents some difficulty as it is evident in the analysis of data compiled for seedlings obtained by back crossing F_1 to the elongate parent 211-V. If a "unit character" condition were prevailing, 50 per cent of these seedlings would have short hypocotyl length and 50 per cent long hypocotyl. The observed results, as presented in Table 13 and analyzed in Table 15, reveal that approximately only one third of the plants have a relatively short hypocotyl section while the remaining seem to belong to the long hypocotyl class. This situation may suggest that more than one pair of factors are operating in the inheritance of hypocotyl elongation in Phaseolus vulgaris. Supposing that two pairs of genes (AA, BB), when present simultaneously in dominant condition are responsible for short hypocotyl but result in gradually longer hypocotyl as the number of dominant alleles decreases while that of their recessive counterparts increases, short hypocotyl and long hypocotyl plants would be in a 9:7 ratio in the F_2 population. The proportion of 207 short hypocotyl to 147 long hypocotyl individuals observed in the latter generation is approximately a 9:7 ratio, as illustrated by a chi-square test in Table 16.

Summing up results obtained for the various crosses that were made, it seems to occur that segregation into short and long hypocotyl sections in any one of these matings nearly expresses the type of recombination that possibly could be expected under a duplicate recessive epistasis hypothesis. If A- B- results in short hypocotyl, and all other combinations produce long hypocotyl the following is true:

1. In F_1: AABB x aabb ⟶ AaBb 100% short
   short long short

2. In BC_s: AABB x AaBb ⟶ AABB, AABb, AaBB, AaBb 100% short
   short long short

TABLE 15. Experiment II (b). Computation of χ^2 for a 3:1 expectation for BC_v.

Class	obs	exp	dev	χ^2
Short	9	10	-1	.100
Long	31	30	+1	.033
Tot. 2	40	40	0	.133

Probability 70 to 95%

Remark: Excellent χ^2 agreement

TABLE 16. Computation of χ^2 for a 9:7 expectation for F_2.

Class	obs	exp	dev	χ^2
Short	207	200	+7	.245
Long	147	154	-7	.318
Tot. 2	354	354	0	.563

Probability 30 to 50%

Remark: Very good χ^2 agreement

3. In BC_v: aabb x AaBb ⟶ AaBb; Aabb, aaBb, aabb 1:3 short to long.
   long short short long

4. In F_2: AaBb x AaBb ⟶ 9 A-B-, 3 aaB, 3 Abb, 1 aabb 9:7 short to long.
   short short short long

Actual results for similar crosses in the present study are not significantly different from the foregoing, and thus the data seem to fit the hypothesis above mentioned. Most geneticists working on related problems agree on assuming a multiple factor hypothesis to explain internode elongation in Phaseolus vulgaris.

A slight discrepancy may be noticed in the distribution of hypocotyl lengths among F_1 and BC_s seedlings, as it has been found that three plants in the former and one plant in the latter generation belong to the 8.5 - 9.4 cm class, which is outside the distribution range of the short parent if two standard deviations are added to its average length to cover 97 per cent of the area of normal distribution. This excess length may be due to hybrid vigor or additive effects of the recessive epistatic factors a and b. Other symbols more appropriate to depict the genes affecting hypocotyl elongation in common beans may be chosen to replace the tentative letters A and B used in the present paper.

Summary and Conclusions

The small figures computed as standard deviations of Sanilac, 211-V, and F_1 relative to their resepctive hypocotyl mean length indicate a tendency of individual measurements to conform closely to a central value. This situation implies that elongation limits of this part of the stem in these varieties of beans are fairly stable and predictable, environmental conditions being equal. Besides, more likelihood is achieved in trying to classify offspring as to hypocotyl length.

Statistical tests carried out in analyzing the data obtained from parents F_1, F_2, and reciprocical back crosses reveal that there is no evidence against the hypothesis that inheritance of hypocotyl elongation in Navy beans (Phaseolus vulgaris) can be explained according to the duplicate recessive epistasis theory. Snyder and David (1957) report that such a case occurs in the transmission of purple color in flowers of the common yellow daisy and in other plant stocks, where single flowers depend upon the simultaneous presence of two different dominant genes, the absence of either or both resulting in double flowers. These authors also mention that in human beings, deaf-mutism appears to be inherited in the same manner but that, nevertheless, duplicate epistasis is more common in plants than in animals.

The above conclusion is a slight modification of that reached by Lamprecht (1948) in a report following a study on the inheritance of the slender-type of Phaseolus vulgaris by crossing dwarf and tall varieties of beans. He remarked that slender is caused by recessivity in two genes for the length of the internodes: Cry, La, each in dominant condition, preventing the length growth of the internodes.

SECTION III

DEVELOPMENTAL STUDIES

Materials and Methods

This experiment was intended to determine whether the difference in hypocotyl elongation between Sanilac and 211-V is due to difference in cell lengths, to difference in cell number, or to both. At the end of the spring, 1960, Foundation seeds of both varieties were sown at the Plant Science Greenhouse of Michigan State University. Twenty-two days after germination, four seedlings of each group of plants were selected and their hypocotyl region cut into four equal parts. Pieces one centimeter long were taken from each one of these parts. After fixation and embedding in paraffin, these sections were sliced to a 10 micron thickness with a microtome, mounted onto slides, and finally triple-stained. For each variety, twelve longitudinal-section slides were prepared by this method.

Results

Upon observation under a compound microscope equipped with a micrometer, the cortical region appeared to be more uniform in the arrangement of the cell layers and to offer more ease in the measurement of the cells than the tissues inward. From each slide, twenty-four cells of the first three cortical layers beneath the epidermis were measured with a precision of one micron under a 125 X magnification. These twenty-four observations were grouped into two sets of twelve measurements each and the mean value of each set recorded.

The results are reported in Table 17 and Plate IV (A, B) illustrate typical cell expansion in both varieties. Under the above mentioned magnification, the average hypocotyl cortical cell length of Sanilac was found to be 11.33 microns and that of 211-V to be 10.92 microns. In Table 18 an analysis of variance carried out for the obtained data shows no significant difference between these two means which represent relative rather than absolute values of the average cell length for the region considered in both varieties.

Discussion of Experimental Results

As the long hypocotyl variety 211-V seems to develop cells even slightly shorter than those of the short hypocotyl type Sanilac, it becomes mandatory that the hypocotylary axis of the former contain more cells than that of the latter, at least for the tissue under consideration. Tentatively an explanation of this increase in cell division rate in 211-V as compared to Sanilac may take into account the possibility of phytohormonal influences. Various growth promoting substances have been extracted from higher plants. Among many instances, lately, in 1958 Radley (20) reported the presence of 0.4 μg (microgram) of Gibberellic Acid (GA) in an extract from 250 gr of tall peas. She also obtained gibberellin-like substances in dwarf bean seed (Phaseolus vulgaris). The same year Macmillan and Suter identified gibberellic substances from immature runner bean seeds as Gibberellin A (13). A

TABLE 17. Experiment III. Cortical cell lengths of Sanilac and 211-V.*

	Sanilac				211-V			
Plant No.	1	2	3	4	1	2	3	4
Section 1	12	9	9	12	9	13	8	13
	13	12	11	11	10	12	9	13
2	14	12	11	9	14	11	8	12
	15	11	13	9	13	11	9	14
3	11	11	12	10	10	10	9	12
	10	11	14	10	12	10	8	12
Average	12.50	11.00	11.67	10.17	11.33	11.17	8.50	12.67
Grand Average	11.33				10.92			

*Lengths expressed in microns with a 125X magnification.

TABLE 18. Experiment III. Analysis of variance for cortical cell lengths of Sanilac and 211-V.

Source of Var.	D.F.	S.S.	M.Sq.	F
Total	47	151.25		
V	1	2.08	2.08	.56 N.S.
P	6	21.58	3.60	.96 N.S.
S	2	6.50	3.25	.87 N.S.
V x S	2	.67	.34	.09 N.S.
P x S	12	30.67	2.60	.67 N.S.
Error	24	89.75	3.74	

V: Variety P: Plant S: Section

N.S.: Not significant at the 5% level.

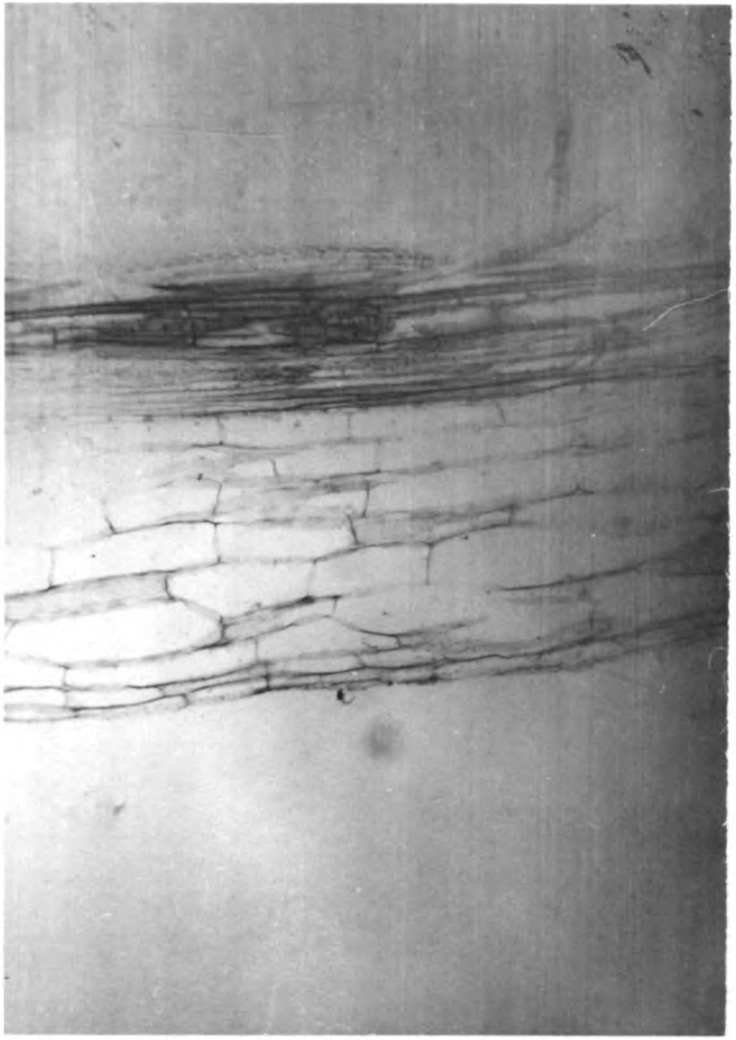


FIGURE 7. Cell development in a hypocotyl section of Sanilac (10X).

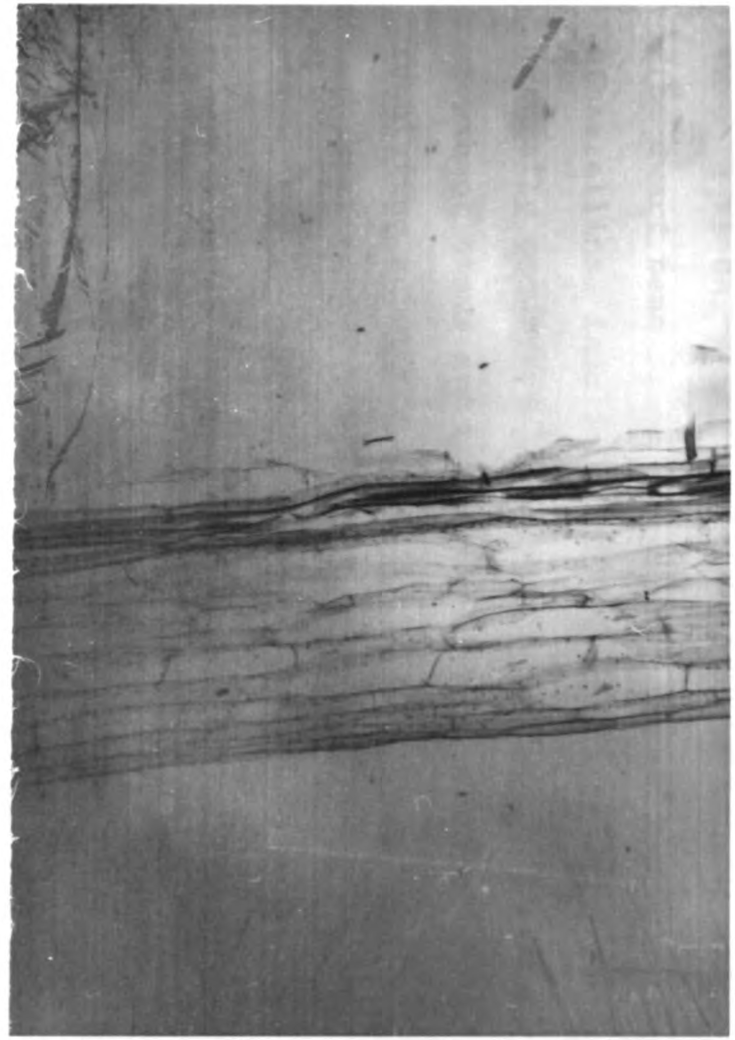


FIGURE 8. Cell development in a hypocotyl section of 211-V (10X).

leaf growth substance, with the same Rf value in isopropano-ammonia as GA, was found in the acid fraction of the extracts from dwarf bean primary leaves and colyledons. Besides the Gibberellins, Indoleacetic acid (IAA) and other auxins naturally occurring in plants have been recognized to promote growth of stems and coleoptiles. Kinetin, another growth-regulating substance, has not been reported as occurring naturally in plant tissues.

As to the specific effects of the various organic growth substances that have been tested so far, contradictory opinions have been expressed. To remain within the scope of this study, only a few of the recent papers published on the subject have been reviewed. Preston and Hepton (19) reached the conclusion that the major growth-regulating effects of most auxins is exercised on the cell wall and that Indoleacetic acid produces a marked increase both in elastic and plastic extensibility on living turgid parenchyma cells short or elongate. The silence of these authors on the effects of IAA upon cell division may suggest that either these effects are nil or insignificant.

Guthridge and Thompson (24) concluded from measurements of cell and petiole lengths in strawberry that cell division and elongation were both induced by GA. Previous evidence, brought up by Greulach and Haesloop (7), also suggested that stems elongate under the influence of Gibberellin more from cell division than from increase in cell size of dwarf bean internodes. This last point seems to be in very good accordance with the observations recorded in the present investigation.

Although action of gibberellin is seen to depend in some extent on external conditions, the current interpretation in phytohormonal studies is toward gibberellin-like rather than auxin-like growth activity.

Summary and Conclusions

Cortical cells of hypocotyl sections of Sanilac and 211-V were measured with a compound microscope equipped with a micrometer for comparison of cell lengths and cell number in the hypocotylary axis of these two varieties of Navy beans. No significant difference was noticed in the mean cell length of the seedlings of both the short and the elongate types of plants for the tissue considered. It was therefore concluded that more cells must be present in the cortex strands of 211-V to account for its longer hypocotyl region as compared to that of Sanilac. Reviewing recent works on phytohormonal influences in higher plants with special reference to *Phaseolus vulgaris*, it is suggested that gibberellic or gibberellin-like substances could be assumed to be responsible for the increase growth rate in the long hypocotyl variety. It is hoped that further studies on phytohormones and their relation to growth and development are successful in opening additional avenues of information regarding this subject.

GENERAL SUMMARY AND CONCLUSIONS

To investigate the basis of the differential hypocotyl elongation of two commercially grown varieties of Navy beans, Sanilac and 211-V, environmental, genetic, and developmental studies were undertaken.

Following application of four combinations of day length and light intensity treatments, it has been observed that although differences in mean hypocotyl length between the short and the elongate types of plants were most pronounced under long day, high light intensity conditions, environmental influences were not solely or primarily responsible for the difference in hypocotyl elongation between Sanilac and 211-V. Intrinsic factors have been assumed to chiefly control growth patterns of the seedlings of these two varieties of beans.

Upon study of the mode of inheritance of the short hypocotyl trait in Sanilac and its counterpart, the long hypocotyl character in 211-V, by means of crosses and back crosses up to the second generation level, no significant evidence was found to rule out the hypothesis that transmission of the hypocotyl elongation trait in Navy bean (Phaseolus vulgaris) could be explained on the basis of the duplicate recessive epistasis hypothesis.

Cytological assays on cortical cell layers of the hypocotylary axis of the seedlings of these two types of bean plants reveal no appreciable difference in cell lengths of both varieties. Such an indication implies the presence of a larger number of cells in a given cortical strand of 211-V than in a corresponding strand in Sanilac. Referring to recent works on phytohormonal activity in higher plants, particularly in the species Phaseolus vulgaris, it has been assumed that gibberellic or gibberellin-like substances might be taken into account in a tentative explanation of the increased cell division rate in 211-V over Sanilac.

The results from the genetic and developmental studies considered together, suggest the following alternative hypotheses:

1. All bean varieties possess gibberellin-like substances which tend to promote hypocotyl elongation, but most varieties like Sanilac also possess a genetic inhibitory system, which, when two non-allelic genes are present in dominant condition effectively suppress the phytohormonal growth action.

2. Only those varieties of beans with recessive alleles in the homozygous state at both the postulated loci involved are able to produce the gibberellin-like material and no direct inhibiting system is present.

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