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Inheritance Studies in Phaseolus Vulgaris

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INHERITANCE STUDIES IN PHASEOLUS VULGARIS[1]

T. M. CURRENCE

The development of the snap bean as a canning crop and as a fresh vegetable has served to emphasize the importance of certain pod characters. The canning trade, especially, is insistent that the pods of a variety meet certain requirements of shape, size, color, tenderness, and uniformity. While a number of genetic studies have been made on the bean plant they have for the most part failed to include pod characters. Where pod characters have been worked with, the results have been somewhat indefinite and unsatisfactory. In an attempt to clarify and extend the genetic information on some of the important characters of the pod, the studies composing this paper were made. The characters chosen are lignified tissue in the sutures and in the side walls, the shape of the pod in cross-section, and color of the pod as related to localized chlorophyll development.

REVIEW OF LITERATURE

A number of workers have reported on the inheritance of strings in the bean pod. The majority have considered the presence of strings to differ by a single genetic factor from the absence of them. Stringlessness has been largely considered the dominant allelomorph. Emerson (1904) submitted data indicating a single-factor relationship in certain crosses, but also data from other crosses which could not be readily explained on this basis if the classifications were properly made. The second type of result reported by Emerson gave an intermediate F_1, and an excess of stringy type in F_2. Progeny of stringless, intermediate, and stringy F_2 plants gave segregation in F_3. In other crosses the stringy character did not segregate in F_3. Tschermak (1916) reported results much the same as those of Emerson's and considered stringlessness dominant. A part of his data could not be explained by a single-factor difference. More recently Wellensiek (1922) reported data that support the single-factor hypothesis but he did not have an F_3 generation to verify his conclusion. His F_2 was grown from three different crosses and was made up of 191 plants. The results closely approximated the three stringless to one stringy expectation. Joosten (1927) reported rather extensive studies of the morphological structure of the string. He stated that the string is composed of a group

[1] This paper was also submitted to the faculty of the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of doctor of philosophy.

Grateful acknowledgment is made to Dr. R. A. Emerson for valuable help in starting this study and for the helpful suggestions given during the course of the work.

of schlerenchymous cells that form the cap of the fibrovascular bundles. A certain positive relationship was found to exist between the degree of lignification of these cells and the strength of the string when removed. Joosten suggested that an entirely stringless variety probably does not exist and that maturity of pods and environment greatly affect the degree of stringiness. He submits data which are not in agreement with the single-factor hypothesis and believes the genetic relationship to be a complex one.

Tschermak (1916) studied the inheritance of pod shape in a cross of round by flat. His data suggested round to be dominant, or nearly so; and a complicated segregation to occur in the F_2 generation.

Woycicki (1927) reported on the inheritance of breadth and thickness of pods and also the ratio between the two characters. The studies were made on two crosses of three different varieties. In both crosses the width was considered as a two-factor difference. In one of the crosses the thickness was also caused by two factors. In the second cross there was not a significant difference between the thickness of the parents, but in F_3, certain lines appeared that apparently were distinctly different for thickness. When the ratio of width to thickness was taken there appeared to be three or four cumulative factors. By segregating different combinations of factors for width and thickness, lines were obtained that were flatter and more round than either parent.

Pod color has been studied by several workers; Emerson (1904) and Tschermak (1916) found the green type dominant to yellow and a ratio approaching three green to one yellow in the second generation. F_3 results also were in agreement with those expected from a single-factor difference. The silver color of the Crystal-White Wax variety has not been studied, altho the variety was used by Halsted (1907) in crossing work. He failed to distinguish the double recessive type from the ordinary yellow, and made no report on the various proportions of the different colors which segregated.

METHODS OF STUDYING THE LIGNIFIED CELLS OF THE POD SUTURES

A microchemical test for lignin seemed to be the most accurate method of determining the presence or the absence of schlerenchymous tissue in the pod sutures. The method of breaking the point of the carpel and attempting to pull out the string has certain advantages, but is somewhat unreliable for making accurate classification. Especially is this true if more than two phenotypes are wanted. The chief objection to using the microchemical test is the time required to cut and examine the sections. This was found to limit the number of plants that could be studied.

The analine sulphate test for lignified cells was found to distinguish very sharply between the stringy and stringless types when applied to the parental material. The test reacts on lignified tissue to give a very bright yellow color to sections from stringy pods. The sections from the stringless parent were stained by the test, but the color was a dark brown and was readily distinguished from that of the stringy parent.

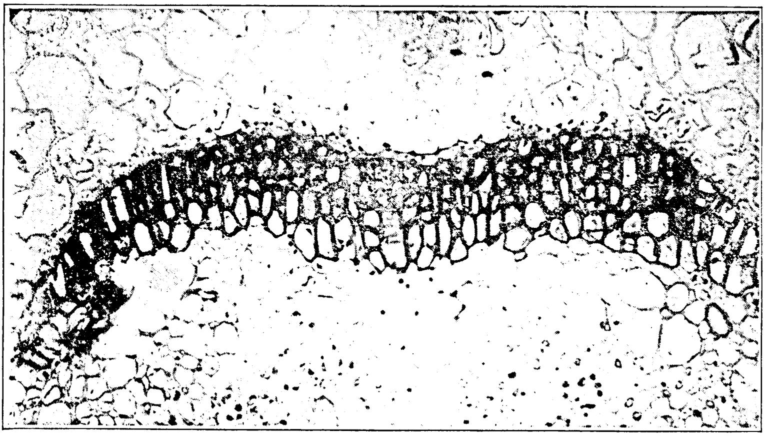

Fig. 1. Transverse Sections of the String Area of the Stringless Type
The groups of dark stained cells are the strings. Enlargement about 280 times.

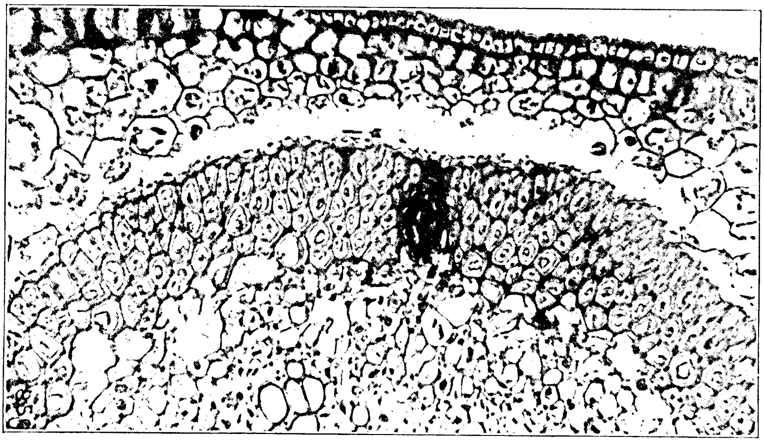

Fig. 2. Transverse Sections of the String Area of the Stringy Type
The groups of dark stained cells are the strings. Enlargement about 280 times.

Anatomically the string appears to be the cap of the fibrovascular bundles in the sutures of the pod. The cap is present in the stringless type of beans but the cells do not take on the thickened lignified nature that characterizes the stringy type. When the hybrids were classified, a single pod was taken as representative of the plant. Tests made on a stringy variety indicated only a slight reduction in variability when two or four pods per plant were used and averaged instead of a single-pod sample. The approximate area of the tissue that gave a positive reaction to the test was measured by an ocular micrometer, and the results were expressed in square millimeters. Stringless plants are considered to be those with an area of less than .005 square millimeter. The terms "stringy" and "stringless," therefore, are used to designate these groups.

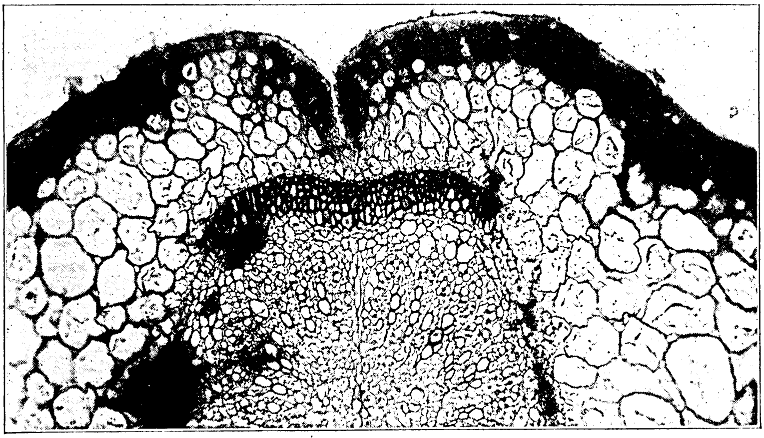

Fig. 3. Lower Magnification of Transverse Sections of the String Area of the Stringless Type

Note fibrovascular bundles located under the strings. Enlargement about 115 times.

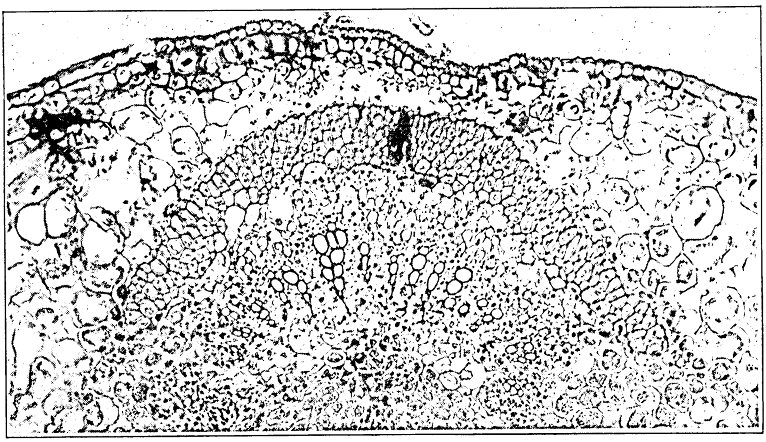

Fig. 4. Lower Magnification of Transverse Sections of the String Area of the Stringy Type

Note fibrovascular bundles located under the strings. Enlargement about 115 times.

The sections were cut from the region between the first and second seeds from the tip of the pod. The measurements mentioned are for half of the string inasmuch as the pods were separated at the sutures and placed in pith to cut the sections. Data from three crosses were obtained through the F_3 generation. These and other crosses will be taken up separately.

A Cross Between Wardwell (Stringless) and Longfellow (Stringy)

The F_1 of this cross consisted of 4 stringless plants and the F_2 was made up of 67 stringless to 33 stringy plants. In determining the breeding behavior of the F_2 plants, the same method of staining and measuring the lignified area was continued. If all the progeny from a single plant gave measurements of less than .005 square millimeter, that plant was considered as breeding true for "stringless." F_2 plants giving all progenies with an area of .005 or greater were considered as homozygous "stringy," and progenies whose measurements included both these groups were classified as being from F_2 plants heterozygous for the string character. The third generation data for this cross are given in Table I.

TABLE I

F_3 PROGENY OF RANDOM SELECTIONS OF F_2 INDIVIDUALS CLASSIFIED AS STRINGLESS AND STRINGY, WARDWELL × LONGFELLOW

Genotype	F_2 stringless plants	F_3		Genotype	F_2 stringy plants	F_3	
		Stringless	Stringy			Stringless	Stringy
Homozygous stringless	1	14	0	Heterozygous	1	2	4
	2	2	0		2	2	3
	3	15	0		3	4	17
	4	12	0		4	13	21
	5	11	0	Homozygous stringy	5	0	8
	6	23	0		6	0	16
	7	17	0		7	0	11
	8	6	0		8	0	9
	9	5	0		9	0	13
	10	12	0		10	0	8
Heterozygous	11	7	2		11	0	10
	12	3	3		12	0	20
	13	2	4		13	0	20
	14	18	4				
	15	5	5				
	16	15	3				
	17	13	7				
	18	8	3				
	19	1	4				
	20	13	16				
	21	14	8				
	22	10	3				
	23	1	11				
	24	3	4				
	25	7	18				
	26	9	4				
	27	3	11				
Homozygous stringy	28	0	10				
	29	0	3				

Twenty-nine progenies were grown from the stringless F_2 group. Ten were stringless, 17 segregated, and 2 were stringy. It should be stated that the ratio of stringless to stringy in the segregating F_3 populations is thought to be of limited significance as the genotypic test of the F_2 shows the error involved in dividing the 2 phenotypes. At least 2 of the F_3 populations from the stringless F_2 group (numbers 2 and 29) are undoubtedly too small to give accurate results. If these 2 lines are excluded, the figures are: 9 breeding true for stringless, 17 segregating, and one breeding true for stringy. If all the 67 F_2 individuals of this class are divided in these proportions, the result is 22.3 homozygous stringless, 42.2 heterozygous, and 2.5 homozygous stringy. As none of the F_3 population from stringy F_2 plants tested were extremely small, they are all included in the calculations. Four of the 13 segregated and the remaining 9 bred true for stringy. This is considered to indicate that 4/13 of the F_2 stringy plants were heterozygous and 9/13 were homozygous stringy. The 33 plants divided in these proportions give 10.2 heterozygous and 22.8 homozygous stringy. These figures combined with those obtained from the stringless give 22.3 plants homozygous stringless, 52.4 heterozygous, and 25.3 homozygous stringy. Altho the phenotypes could not be finely divided, the results from testing the genotypes suggest a single genetic-factor difference. This hypothesis calls for the three genotypes to be present in F_2 in a ratio of 1:2:1; or in this case, 25 homozygous stringless, 50 heterozygous, and 25 homozygous stringy. The P value for the results secured is .84 which means a chance deviation as great as this would be expected eighty-four times in one hundred such trials.

A Cross Between Pencil Pod (Stringless) and Robust (Stringy)

The F_1 of this cross consisted of 6 plants, all of which were stringless. In F_2 there were 157 stringless and 87 stringy plants. Table II gives the F_3 results. Progenies from 10 stringless F_2 plants were grown. Five were entirely stringless and 5 were apparently from plants heterozygous for strings. Dividing the 157 F_2 stringless plants in this proportion gives 78.5 homozygous stringless and 78.5 heterozygous.

Fourteen stringy F_2 plants gave progenies the following generation. Five were homozygous stringy and 8 heterozygous. The 87 stringy F_2 plants in this proportion are 37.3 and 49.7, respectively. Figures for the two F_2 groups combined show the 3 genotypes to be 78.5 homozygous stringless; 128.2 heterozygous; 37.3 homozygous stringy. This is quite a wide deviation from a theoretical ratio of 1:2:1. It may be explained, however, by the possibility that certain of the F_3 lines may be wrongly classified. That this possibility is not a remote one may be noted from the F_3 results given in Table II. If larger F_3 populations were available, certain of the lines would probably be found in other groups.

TABLE II

F_3 PROGENIES OF RANDOM SELECTIONS OF F_2 INDIVIDUALS CLASSIFIED AS STRINGLESS AND STRINGY, PENCIL POD × ROBUST

Genotype	F_2 stringless plants	F_3 Stringless	F_3 Stringy	Genotype	F_2 stringy plants	F_3 Stringless	F_3 Stringy
Homozygous stringless	1	6	0	Heterozygous	1	3	3
	2	13	0		2	22	19
	3	7	0		3	2	3
	4	7	0		4	5	21
	5	7	0		5	4	11
Heterozygous	6	8	7		6	10	6
	7	21	7		7	7	14
	8	15	1		8	5	7
	9	31	15	Homozygous stringy	9	0	23
	10	47	9		10	0	32
					11	0	28
					12	0	12
					13	0	23
					14	0	14

Different classification of a very few lines would greatly change the results, as each group is represented by a small number of F_3 populations.

	Homozygous stringless	Heterozygous	Homozygous stringy
Observed	78.5	128.2	37.3
Calculated 1:2:1	61	122.0	61.0
Difference	17.5	6.2	—23.7

A Cross Between Refugee Wax (Stringless) and Tennessee Green Pod (Stringy)

The data from this cross appear to be markedly different from the two previous crosses, and cannot be readily explained by a single-factor hypothesis. The F_1 of this cross is distinctly stringy; an excess of stringy also occurs in the F_2 grouping. The F_2 population was made up of 19 stringless and 64 stringy. The F_1 consisted of 3 stringy plants. As indicated in Table III, 11 stringless F_2 plants produced progeny—4 were entirely stringless and 7 segregated into both types. Of the 37 stringy F_2 plants that produced progenies in F_3, 19 were heterozygous, 17 bred true for stringy, and one produced only stringless. The figures are considered to indicate that the 83 F_2 plants were made up of the 3 genotypes in the following proportions: 8.3 homozygous stringless, 44.3 heterozygous, and 29.4 homozygous stringy. The hypothesis which may best explain these figures calls for theoretical numbers of 5.2 homozygous stringless, 41.5 heterozygous, and 36.3 homozygous stringy. The P value is approximately .19. Altho this value is rather low to be considered generally significant, the nature of the present data are such that it may be of importance. Small populations and possible errors in classifying the material might tend to detract from the agreement between the theoretical and actual ratios.

TABLE III

F_3 PROGENIES OF RANDOM SELECTIONS OF F_2 INDIVIDUALS CLASSIFIED AS STRINGLESS AND STRINGY, REFUGEE WAX × TENNESSEE GREEN POD

Genotype	F_2 stringless plants	F_3 Stringless	F_3 Stringy
Homozygous stringless	1	11	0
	2	5	0
	3	12	0
	4	4	0
Heterozygous	5	7	4
	6	10	1
	7	20	4
	8	3	1
	9	11	7
	10	13	6
	11	5	2

Genotype	F_2 stringy plants	F_3 Stringless	F_3 Stringy
Homozygous stringless	1	8	0
Heterozygous	2	3	5
	3	3	7
	4	3	1
	5	4	4
	6	3	4
	7	7	4
	8	1	7
	9	4	10
	10	5	8
	11	1	7
	12	11	4
	13	3	8
	14	1	4
	15	4	11
	16	1	8
	17	7	1
	18	3	23
	19	1	15
	20	1	11
Homozygous stringy	21	0	6
	22	0	10
	23	0	7
	24	0	16
	25	0	11
	26	0	8
	27	0	2
	28	0	7
	29	0	11
	30	0	13
	31	0	14
	32	0	12
	33	0	5
	34	0	14
	35	0	13
	36	0	18
	37	0	17

The hypothesis for explaining the data from the 3 crosses assumes one dominant factor producing stringless, but a second factor, when present, inhibits the action of this factor. The factor for stringless is indicated by S and the inhibiting factor is termed T. Assuming these 2 factors it is possible to have 3 genetic types of stringy, namely TTSS, TTss, and ttss; whereas the only possible stringless is ttSS. A cross of

TTss by ttSS would give a phenotypic ratio of 13:3 or a genotypic one of 1:8:7. This is a possible combination for the Refugee Wax × Tennessee Green Pod cross. A cross of ttSS by ttss would give results such as those obtained in the first two crosses discussed.

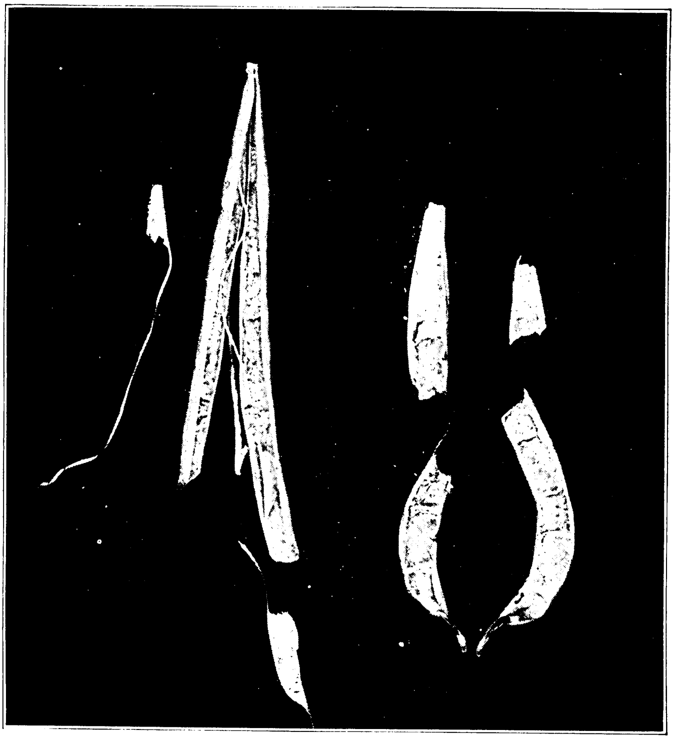

Fig. 5. Pods of the Stringy and Stringless Types

Emerson's data (1904) which did not seem to conform to the hypothesis of a single dominant factor for stringless may possibly be brought into agreement with the data of this paper. Emerson's data for the cross wherein he found dominance to be incomplete are as follows: 10 F_3 lines were grown from stringless F_2 plants; 5 of these bred true and 5 segregated; 7 of the intermediate F_2 class were tested and all were found to segregate; 11 stringy F_2 plants produced progeny; 7 of these bred true for stringy and 4 were heterozygous. Applying these proportions to the F_2 of Emerson's 5 crosses with a population of 114 stringless, 80 intermediate, and 78 stringy, gives the following:

	Homozygous stringless	Heterozygous	Homozygous stringy
Observed	57	165	50
Calculated 1:2:1	68	136	68
Difference	—11	29	—18

P value equals .001.

Thus Emerson's data may roughly agree with the expected results if the crosses were between TTSS and ttSS. That the agreement between the actual and theoretical is very poor is realized, but the difficulties of classifying the material and the combining of data from 5 different crosses are of importance in this connection. There is an important possibility that some of these crosses were between TTss and ttSS. This combination would give dihybrid results and therefore might cause the poor agreement. Unfortunately, the data from Emerson's individual crosses are not available.

The figures in Table IV from a number of additional crosses provide some evidence that may be of interest. These data were obtained by pulling out the string of mature pods and classifying them as either stringless or stringy. Altho the value of these figures is limited by the fact that only phenotypic ratios are available, one point is rather definitely demonstrated and at least one other is suggested. It is fairly definitely shown that the stringlessness of the variety Refugee Wax is genetically different from that of certain other varieties and it is suggested that two genetic types of stringy may also exist.

TABLE IV

F_1 AND F_2 DATA ON THE INHERITANCE OF STRINGS FROM CROSSES BETWEEN VARIOUS VARIETIES

Varieties crossed	Designation number of cross	F_1	F_2 Stringless	F_2 Stringy	Possible theoretical ratio	Deviation
Burpee's Stringless × Davis Wax (stringy)	1	2 stringless	21	7	3:1	0
Burpee's Stringless × Black Valentine (stringy)	2	2 stringy	57	69	7:9	1.9 ± 3.76
Refugee Wax (stringless) × Longfellow (stringy)	3	Unknown	15	47	1:3	0.5 ± 2.30
Refugee Wax × Black Valentine	4	3 stringy	7	17	1:3	1.0 ± 1.43
Refugee Wax × Crystal White Wax	5	2 stringy	13	80	3:13	4.47 ± 2.54
Crystal White Wax × Refugee Wax	6	Unknown	26	152	3:13	7.4 ± 3.51

A single Black Valentine (stringy) plant was crossed with the two stringless varieties, Burpee's Stringless Green Pod and Refugee Wax. As indicated in Table IV, the result approaches a ratio of 7 stringless to 9 stringy in one case and in the other the observed result closely agrees with a 1:3 ratio. The crosses referred to are numbers 2 and 4. It is believed that the difference is too great to be explained on the basis of inaccurate classification of the F_2 plants. This is in disagreement with the theory used to explain the F_3 results, previously discussed, inasmuch as that hypothesis required that all stringless plants be of the same genetic constitution for stringless. It is apparent that the two ratios in Table IV may be explained by assuming two dominant complementary factors necessary for the development of strings. It may be considered that Black Valentine carries both factors, Refugee Wax one, and Burpee's Stringless Green Pod neither of them. But to bring this hypothesis into agreement with results from other crosses requires the assumption of at least two additional factors. Such an explanation is probably too theoretical to be justified without more extensive data than is now available.

That two genetic types of stringy exist is suggested by crosses 1 and 2. Burpee's Stringless was used as the stringless parent in both crosses and gave quite different results with the two different stringy varieties. The divergence is so great between the two results that it is thought a different genetic constitution for the two stringy parents affords the most likely explanation. The fact that the same Burpee's Stringless plant was not used involves the possibility that two genetic type of stringless were present rather than two stringy types.

In the preceding material the author has desired especially to indicate the complex inheritance of the character under consideration. There remains the question of the effect that environment may have on the development of the string. Altho no formal data have been collected on this, a number of stringless and stringy varieties have been observed growing in different localities and under varying conditions. No suggestion of a difference in stringiness or stringlessness which could be correlated with the growing conditions has been noted.

Inasmuch as lignification of the string does not start until the pod has developed for about ten days, the age of the pod has some relation to stringiness in the stringy varieties. After lignification starts it appears to gradually increase until the pod matures.

The indications for a complex inheritance of strings may be summarized by observing the variable results secured from different crosses. In certain of these, stringless is dominant and probably the result of a single gene. In other crosses the dominance is reversed, requiring the presence of two factors for a suitable explanation. The two factors have given indications of being complementary. In one cross the two-factor hypothesis postulates that one factor inhibits the action of the other or is epistatic to it. Two types of stringless are shown to exist and probably the same is true for stringy.

INHERITANCE OF FIBER IN THE SIDE WALL OF THE POD

Fiber in the side wall of the pod is a character of distinct economic importance and one that is also very difficult to study genetically. The difficulty arises from the variable expression of the character at different ages of the pod and probably under different growing conditions.

Furthermore, the lignified tissue in this case occurs so that no suitable method of quantitatively measuring it is readily available. A method of making a chemical analysis of the crude fiber of the pod after the string has been removed is now being tried. However, the data so far obtained with it are not sufficient to be included. The data presented here were obtained by examining pods from individual plants and attempting to classify them according to the parental material. The figures are unsatisfactory and are only presented with the hope that they may be of some interest to others working with this character. Two crosses were studied, Longfellow × Wardwell and Pencil Pod × Robust. The Longfellow and Pencil Pod varieties are comparatively free from fiber in the side wall while the others are very fibrous and tough. F_2 results from Pencil Pod × Robust are as follows:

	Fiberless	Fibrous
Observed ratio	34	132
Calculated ratio 3:13	31.1	134.9
Difference	2.9 ± 3.39	
Calculated ratio 1:3	41.5	124.5
Difference	7.5 ± 3.76	


Fig. 6. Fibrous and Fiberless Pods. Enlargement about 2 times

The F_1 of this cross was distinctly fibrous. The F_2 results closely agree with the theoretical 3:13 ratio. They may also be considered to represent a 1:3 proportion. The data secured by growing progenies from some of the F_2 plants are presented in Table V.

Progenies were grown from 4 F_2 plants that were classified as having fiberless pods. Two bred true for "fiberless" but the total number of F_3 plants obtained from both was only 15. One of the 4 lines appeared to be heterozygous giving 3 fiberless to 13 fibrous F_3 plants. The fourth progeny was made up of 7 fibrous-podded plants. Obviously, the number of lines tested was too small to give an accurate test for the genotype of the fiberless F_2 group. If the 34 plants in this group are divided according to F_3 results, the following numbers are obtained: 17 homozygous "fiberless," 8.5 heterozygous, and 8.5 homozygous "fibrous."

TABLE V

F_3 PROGENY OF RANDOM SELECTIONS OF F_2 INDIVIDUALS CLASSIFIED AS FIBERLESS AND FIBROUS, PENCIL POD × ROBUST

Genotype	Designation number	F_2	F_3	
			Fiberless	Fibrous
Homozygous fiberless	80	Fiberless	9	0
	2	do	6	0
Heterozygous	260	do	3	13
Homozygous fibrous	78	do	0	7
Homozygous fiberless	11	Fibrous	12	0
Heterozygous	235	do	10	17
	52	do	6	22
	100	do	4	11
	83	do	4	18
	9	do	3	6
Homozygous fibrous	121	do	0	47
	10	do	0	42
	41	do	0	31
	113	do	0	14
	118	do	0	52
	45	do	0	23
	38	do	0	27
	4	do	0	18
	28	do	0	16
	102	do	0	14
	93	do	0	12
	53	do	0	5
	89	do	0	12
	6	do	0	6

Twenty fibrous-podded F_2 plants produced progenies the following generation. Fourteen gave only fibrous and a total of 323 plants. Five of the lines segregated into the two types, producing a total of 20 fiberless-podded plants to 74 fibrous-podded ones. The theoretical numbers for a single-factor ratio are 23.5 to 70.5, respectively. The difference is 3.5 ± 2.83. If the F_2 is a 3:13 ratio, the heterozygous plants should give progenies showing a 1:3 and a 3:13 ratio in the proportion of 2:4, respectively. Unfortunately, however, the F_3 populations are too small to distinguish the two ratios. The one fibrous-podded F_2 yet to be considered gave a total of 12 fiberless-podded F_3 plants. On the basis of their breeding behavior the 132 fibrous F_2 individuals then may be of the following genotypes: 92.4 homozygous fibrous, 33.0 heterozygous, and 6.6 homozygous fiberless. Combining these figures with those calculated from the fiberless-podded F_2 progenies gives the following:

	Homozygous fiberless	Heterozygous	Homozygous fibrous
F_2 fiberless	17.0	8.5	8.5
F_2 fibrous	6.6	33.0	92.4
Total	23 6	41.5	100.9

Altho the results are not readily explained on a genetic basis, they suggest the difficult and complex nature of the character.

The data secured on the F_2 and F_3 of another cross are entirely different, thereby giving further evidence that the genetics of the character is complex and differs in different varieties. This cross is that made between Wardwell (fibrous) and Longfellow (fiberless). The F_1 was fibrous and the F_2 agrees fairly well with a theoretical one to three ratio. The data for F_2 were 27 fiberless to 68 fibrous, the expected for a 1:3 being 23.75 to 71.25, respectively. The difference is 3.25 ± 2.85.

But here again the F_3 results (Table VI) do not agree with the expected for a single-factor difference. Progenies were grown from 17 fiberless-podded F_2 plants. Ten bred true giving a total of 128 individuals in F3. The progeny from one plant was entirely fibrous-podded and consisted of 6 plants. The remaining 6 were composed of fiberless and fibrous-podded plants. The numbers of each type were 42 fiberless and 58 fibrous; a very poor agreement with the results expected if the character is associated with a single genetic unit.

TABLE VI

F_3 PROGENIES OF RANDOM SELECTIONS OF F_2 INDIVIDUALS CLASSIFIED AS FIBERLESS AND FIBROUS, WARDWELL × LONGFELLOW

Fiberless F_2				Fibrous F_2			
Genotype	Designation number	Distribution in F_3		Genotype	Designation number	Distribution in F_3	
		Fiberless	Fibrous			Fiberless	Fibrous
Homozygous fiberless	1-5	11	0	Homozygous fiberless	3-19	11	0
	3-1	9	0		3-24	20	0
	3-5	13	0		1-6	16	0
	3-8	11	0		1-17	18	0
	3-27	12	0		3-16	20	0
	3-32	11	0		3-18	22	0
	4-5	16	0		4-11	17	0
	4-6	13	0	Heterozygous	3-14	3	18
	4-18	23	0		3-15	3	10
	4-21	9	0		1-13	1	3
Heterozygous	1-2	7	13		3-28	10	4
	1-3	2	3		3-30	6	6
	3-2	6	4		4-2	5	19
	3-9	21	13		4-14	7	1
	3-11	4	16		4-15	6	2
	4-35	2	9		4-16	2	8
Homozygous fibrous	4-24	0	6		3-17	1	13
					4-8	7	21
				Homozygous fibrous	1-8	0	10
					1-22	0	8
					3-7	0	6
					4-25	0	5
					4-26	0	3

Twenty-three fibrous-podded F_2 individuals produced progenies in F_3. Seven of these populations were fiberless, 11 were heterozygous, and 5 had only fibrous-podded plants. The seven with only fiberless had a total of 124 F_3 individuals. The eleven segregating lines had 51 plants without and 105 with fiber. The expected numbers from individuals heterozygous for a single factor are 39 and 117, respectively, the difference between the actual and the theoretical being 12 ± 3.80.

The 5 populations which were entirely fibrous were made up of 32 plants. All are extremely small populations and, therefore, of limited value. It should be noted also that the single plant classified as fiberless in F_2 that gave nothing but fibrous-podded F_3 plants produced a very small population consisting of only 6 individuals.

On the basis of the F_3 figures, it is possible to separate the F_2 phenotypes into the three genotypes considered and to calculate the number in each genotype. The 27 fiberless-podded F_2 and the 68 fibrous should be composed of the following:

	Homozygous fiberless	Heterozygous	Homozygous fibrous
F_2 fiberless	15.9	9.54	1.59
F_2 fibrous	20.7	32.52	14.75
Total	36.6	42.06	16.34

The result is not readily explained without assuming a number of factors that again might not be justified with such small numbers. The above ratio in comparison with a 1:2:1 and a 7:8:1 gives P values that are infinitely small.

Therefore, it is apparent that altho the F_2 ratios give indications of one or two factor differences, the indications are not supported by genotypic tests. Two difficulties which one must consider when studying the inheritance of this character are outstanding. The problem of properly classifying the phenotypes is perhaps the more serious. Altho chemical analysis for crude fiber is a slow and expensive procedure to use for genetic classifications, it may be a means of securing accurate data. The second difficulty is that of securing a large number of seeds from individual plants. This may be overcome to some extent by giving the plants the most favorable growing conditions possible. Also using pole or climbing varieties may be desirable as they are more productive of seed.

INHERITANCE OF WIDTH OF PODS

The varieties which were used for the cross are Wardwell, a wide, flat-podded variety, and Longfellow, a narrow, round-podded type. The difference between the shape of the pods of the two varieties is one of width from suture to suture. This has been demonstrated by measurements of the thickness of the pods on both varieties. No statistically significant difference in thickness was shown. In the F_2 populations so far studied there has not been a significant correlation between pod

TABLE VII

MEANS AND COEFFICIENT OF VARIABILITY FOR POD WIDTHS OF PARENTAL F_1, F_2, AND F_3 GENERATIONS, WARDWELL × LONGFELLOW

Generation	Classes for mean width of pod in centimeters							Total lines	Coefficient of variability classes
	0.9	1.0	1.1	1.2	1.3	1.4	1.5		
P_1		1						1	6
P_1							1	1	9
F_1					1			1	3
F_2				1				1	17
F_3 lines			1					1	0
								0	1
								0	2
								0	3
		2	2		3		1	8	4
	1		2	2	2			7	5
		2	3	1	2			8	6
	1	1	2	2		1		7	7
		2	1	1				4	8
					1			1	9
			1	2	2			5	10
				1				1	11
				2				2	12
			1					1	13
				1	1			2	14

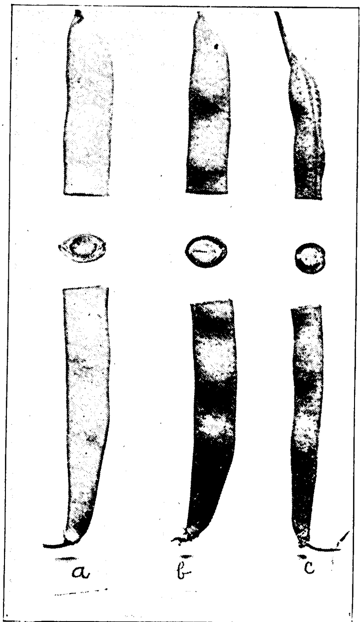

Fig. 7. Representative Pods from Parents a, c, and F_1 b. Note intermediate shape and width of b.

thickness and the ratio of pod width to pod thickness. The varieties have proved to be distinctly different in width measurements and width has shown a high degree of correlation to the above-mentioned index.

The data obtained are given in Tables VII and VIII. It will be noted that one cross was carried through the F_3 generation and another through the F_2.

The figures were secured by measuring the width of not less than four pods per plant. The average of the four pods was considered to represent the pod width for that particular plant. Plants of the parent varieties were grown in comparison with the F_2 hybrids of both crosses, and with the F_1 of the second cross. The F_1 and F_3 of the first cross were grown without parental material for comparison.

TABLE VIII

RESULTS SECURED FROM THE SECOND CROSS OF WARDWELL × LONGFELLOW

Generation	Year grown	Width classes in centimeters 0.6	0.7	0.8	0.9	1.0	1.1	1.2	Mean	Standard deviation	Coefficient of variability
P_1	1928	18	31	3					.671 ± .006	.060 ± .004	9.00 ± .59
P_1	1929		15	36	1				.773 ± .005	.056 ± .004	7.23 ± .48
P_1	1928						9	6	1.140 ± .011	.063 ± .008	5.52 ± .68
P_1	1929						6	15	1.171 ± .008	.053 ± .005	4.52 ± .47
F_1	1928				7	3			.903 ± .012	.055 ± .008	6.10 ± .92
F_2	1929	5	18	39	40	24	1		.850 ± .007	.120 ± .005	14.12 ± .60

In both crosses the F_1 populations were small but appeared to be intermediate for pod width and were no more variable than the parents.

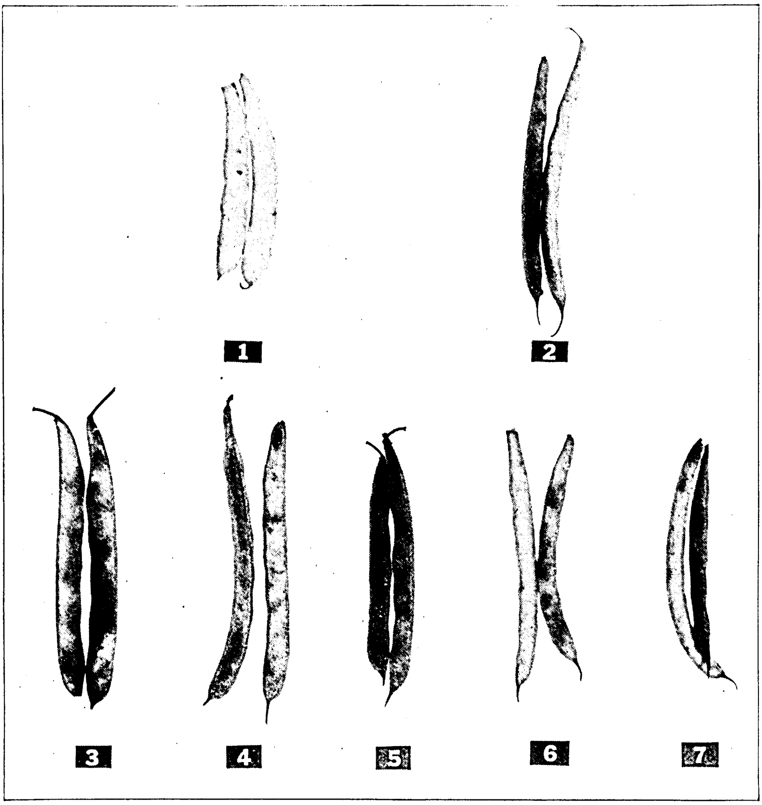

Fig. 8. Representative Pods from Parents 1, 2, and Various F_2 Classes 3 to 7

Altho the F_2 means are intermediate, the variation is quite distinct. The F_3 results are not satisfactory owing to the small populations and to the absence of parental material for comparisons. There is some evidence that certain lines from all the F_2 groups were fairly homozygous in their F3 behavior. This is a possible indication that the character is controlled by a number of factors. Table IX shows a correlation surface for the width of F_2 pods on plants heterozygous for the character and the mean pod width of their F_3 families. These heterozygous plants were selected on the basis of the variability which they showed in F_3. The fact that the correlation is significant is an indication that more than one factor pair is concerned in the development of pod width. If only a single pair were concerned, the probabilities are that all heterozygous F_2 individuals would give approximately the same means in F_3.

The correlation between all F_3 means and their F_2 measurements is given in Table X. The high degree of correlation in this case is believed to indicate an important rôle for inheritance in the development of this character.

TABLE IX

RELATION OF POD WIDTH ON SEGREGATING F_2 PLANTS AND MEAN WIDTH OF THEIR F_3 FAMILIES

Width of segregating F_2 plants	Classes for mean width in F_3, in centimeters: 1.00	1.05	1.10	1.15	1.20	1.25	1.30	1.35	Total
1.0	2	1							3
1.1			1						1
1.2		1	4						5
1.3			2	2		1			5
1.4					1				1
1.5				1	1				2
1.6								1	1
Total	2	2	7	3	2	1		1	18

$r = .601 \pm .102$

TABLE X

RELATION OF WIDTH OF POD ON F_2 PLANTS AND MEAN WIDTH OF F_3 FAMILIES—WARDWELL × LONGFELLOW

Width of F_2 pods	Classes for mean width in F_3, in centimeters: 0.95	1.00	1.05	1.10	1.15	1.20	1.25	1.30	1.35	1.40	1.45	Total
1.0	1	1	4	4	1							11
1.1		1	1	2	1							5
1.2	1	1	2	1	6		1	2				14
1.3					3	1	3	1				8
1.4						1	2					3
1.5		1				1		1		1	1	5
1.6									1			1
Total	2	4	7	7	11	3	6	4	1	1	1	47

$r = .66 \pm .056$

LINKAGE RELATIONS

The cross between the Wardwell and Longfellow varieties provided an opportunity to observe the linkage relations for string, for fiber, and for pod width. Altho no definite genetic relationship was indicated between string and the other characters, there is evidence that width of pod is associated with the development of fiber. The F_2 data of the second cross between the above named varieties is given in Table XI. The Wardwell variety is wide and fibrous, whereas Longfellow is narrow and comparatively free of fiber. If the characters tend to remain together, the F_2 plants with fiber should have a greater pod width than those without it. The data show that this actually occurs. The difference between the means is several times greater than its probable error and is, therefore, statistically significant.

Further evidence of this linkage is afforded by a number of varieties of snap beans. No wide-podded variety has been observed that did not develop fibrous tissue in the side walls. Likewise a fibrous round-podded variety is unknown to the writer. Inquiries on this point have been put to several gardeners and scientific workers. In no case has an exception to the above generality been indicated.

One question of interest in this connection is whether both characters are dependent on the same gene or genes or on different ones closely linked in the same chromosome. The F_2 material which was grown in 1929 was arbitrarily separated into narrow and wide pods. Where these were classified for the presence or absence of fiber, all the narrow pods, with but one exception, were noted as being free of fiber.

TABLE XI

COMPARISON OF THE WIDTHS OF PODS THAT DEVELOP FIBER WITH THOSE THAT DID NOT DEVELOP THIS TISSUE

	Width, centimeters						Mean
	0.7	0.8	0.9	1.0	1.1	1.2	
Fibrous		2.0	15.0	25.0	22.0	1.0	1.004 ± .007
Fiberless	5.0	17.0	22.0	22.0	2.0		0.882 ± .007

In the case of the wide pods there were only four found to be without fiber. It has already been suggested that fiberless and fibrous pods are often difficult to distinguish accurately. Therefore, these five possible crossover types may have been wrongly classified for fiber. It seems quite probable that the presence of fiber in the side wall of the pods might cause them to develop the flat, wide type rather than the round, fleshy type. A definite answer to this question may result from the F_3 results of the F_2 of 1929. The F_3 records of the previous cross between these varieties were lost before this particular point was checked. If it can be shown definitely that the two characters are controlled by the same genes, it may be of considerable interest, as it would facilitate the detecting of fibrous pods, and thereby genetic studies of this character.

In concluding the discussion on pod width it can be stated that between the varieties crossed there is undoubtedly a difference of at least two factors. Also pods with fibrous side walls tend to show a greater width than those that are free of such fiber. This may be the result of either a genetic linkage or of multiple allelomorphs.

STUDIES ON POD COLOR

The variety known as Crystal White Wax produces pods of an unusual color. The color is a grayish or silvery one that is readily distinguished from the green and yellow pod colors of the better known varieties. So far as known the inheritance of this color has not been previously investigated.

Tschermak, Emerson, and others have shown yellow to be recessive to green and to differ from it by a single factor. If the silver color of Crystal White Wax also differs by a single factor from green and is not allelomorphic to or linked with the factor for yellow, a cross of silver by yellow would be expected to show a dihybrid segregation. Several crosses involving these various colors have been made. A cross between Wardwell (yellow) and Crystal White Wax (silver) has been carried through the F_3 generation and will be discussed in detail. The F_1 generation from this cross was made up of four green-podded plants. In the F_2 generation, a new color type was segregated as the pure recessive. This is a clear white pod of unusual attractiveness. The two parental colors and green were also recovered in F_2.

According to the description given by Joosten (1927) of the anatomy of the bean pod in cross-section, the color of the green pod is located uniformly in both the inner and outer parenchyma. Altho the yellow type has green plastids when the pods are young, these change to yellow as the pods develop. However, the inner parenchyma of the yellow pod, so far as observed, never appears to contain colored plastids. The silver type has a reduced number of green plastids in the inner parenchyma, and in the outer parenchyma appears to have no color except a narrow band of green adjoining the fibrovascular bundles in this tissue. The white, being a combination of the factors for yellow and silver, is of most interest. It has the colorless inner parenchyma of the yellow type and the colorless outer parenchyma of the silver. The ring of green of the silver adjoining the fibrovascular bundles is present but is greatly reduced in extent.

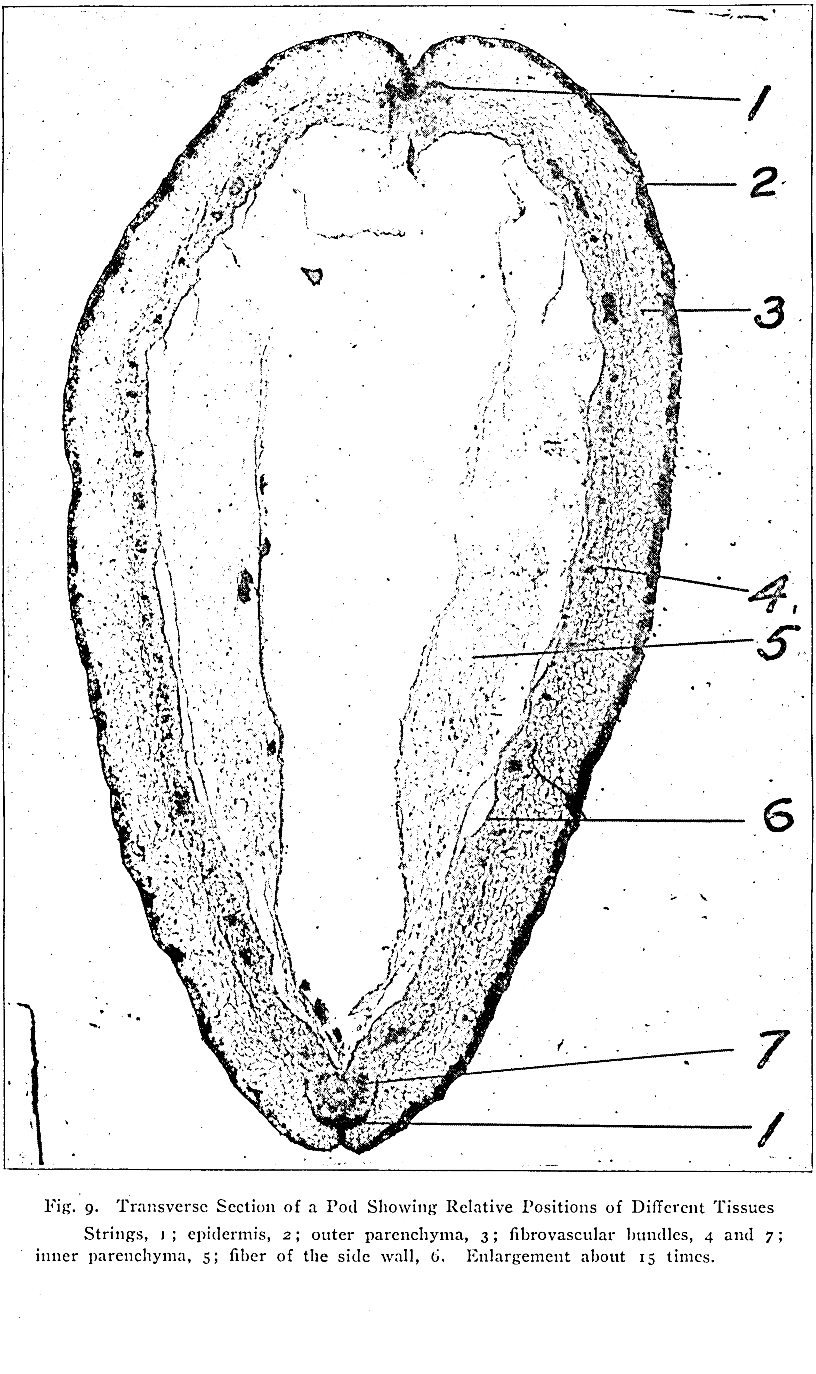

Fig. 9. Transverse Section of a Pod Showing Relative Positions of Different Tissues

Strings, 1; epidermis, 2; outer parenchyma, 3; fibrovascular bundles, 4 and 7; inner parenchyma, 5; fiber of the side wall, 6. Enlargement about 15 times.

The F_2 plants were made up of the following colors:

	Color types			
	Green	Yellow	Silver	White
Observed	96	22	24	8
Calculated 9:3:3:1	84	28	28	9
Difference	12	−6	−4	−1

The P value calculated from the above figures on the basis of a 9:3:3:1 ratio was found to equal .29 or over one chance in four of getting this difference as a result of random sampling.

Thirty of the F_2 green plants were selected at random for testing in F_3. On the basis of the dihybrid hypothesis these green-podded plants would be expected to show four different genotypes as follows:

Number	Genotype	Expected progenies
1	AABB	All green
2	AaBB	3 green, 1 yellow
2	AABb	3 green, 1 silver
4	AaBb	9 green, 3 yellow, 3 silver, 1 white

Three of the total of 30 plants bred true for green pods; 5 segregated for green and yellow, 9 for green and silver, and 13 for green, yellow, silver, and white. Comparison of the observed with the calculated follows:

	Color types			
	Green	Yellow	Silver	White
Observed	3.0	5.0	9.0	13.0
Calculated 1:2:2:4	3.3	6.7	6.7	13.3
Difference	—0.3	—1.7	2.3	—0.3

The P value for these figures is 0.74.

The 13 plants segregating for 4 colors would be expected to give a ratio of 9:3:3:1 or a duplicate of the F_2 results. The observed and expected results from the 13 plants follow. The P value was calculated from these data to be .62.

	Color types			
	Green	Yellow	Silver	White
Observed	288	94	82	30
Calculated 9:3:3:1	277	93	93	31
Difference	11	1	—11	—1

Five F_2 green-podded plants gave only green and yellow in F_3. The total number of F_3 individuals was 160, of which 117 were green an 43 were yellow; the expected numbers are 120 and 40, respectively. The deviation therefore is 3 ± 3.69.

Nine F_2 green-podded plants produced only green- and silver-podded individuals in F_3, the total number being 361. Of these, 275 were green- and 86, silver-podded; the expected numbers are 271 and 90, respectively. The deviation is 4 ± 5.55.

Progenies from 10 F_2 yellow-podded plants selected at random were grown in F_3. These F_2 plants were expected to show 2 genotypes in the proportion of one homozygous for yellow to 2 heterozygous for yellow and white. Seven of the 10 plants produced both yellow- and white-podded individuals in F_3, while 2 produced only yellow-podded ones, and one produced green- and yellow-podded individuals. Very probably this individual bore green pods and was wrongly classified in the F_2 records. When a green-podded plant reaches maturity, the chlorophyll gradually disappears from the pods at which time such a plant may readily be mistaken for a yellow-podded one. The F_3 results are approximately those which would be expected from a plant heterozygous for green and yellow. Disregarding this line, the 9 yellow-podded F_2 plants give a deviation from the theoretical genotypic ratio of 1 ± 0.95.

The F_2 yellow-podded plants producing both yellow and white pods in F_3 gave a total of 181 individuals. Of these 137 were yellow and 44 were white; the expected numbers were 136 and 45, respectively. The deviation is equal to 1 ± 3.93. The two plants that gave only yellow-podded individuals produced a total of 129 F_3 plants. The one individual which gave green and yellow produced 10 green to 4 yellow. This is a close approximation to the ratio expected from a green plant heterozygous for green and yellow.

Progenies also were grown from 10 of the F_2 silver-podded plants. They would be expected to show the same type of behavior as the F_2 yellow-podded individuals. Six of the 10 gave silver and white, and 4 gave only silver; the expected numbers are approximately 7 and 3, respectively. The deviation is 1 ± 1.0. The total number of plants obtained in the F_3 generation from the segregating F_2 individuals was 151, of which 107 were silver and 44 white. The theoretical numbers are 113 and 38, making the deviation equal to 6 ± 3.59.

Six progenies were grown from plants that bore white-colored pods in F_2. The total number of F_3 individuals grown was 136, all of which bore only white-colored pods.

Two additional crosses between yellow- and silver-podded plants were carried through the F_2 generation. A cross of Refugee Wax by Crystal White Wax yielded 2 green-podded F_1 plants. The F_2 of this cross gave the following:

	Color types			
	Green	Yellow	Silver	White
Observed	55.0	13.0	14.0	4.0
Calculated 9:3:3:1	48.6	16.2	16.2	5.4
Difference	6.4	—3.2	—2.2	—1.4

The P value for these figures is 0.56.

From the second cross, which is the reciprocal of the above, 3 green-podded F_1 plants and the following F_2 numbers were obtained:

	Color types			
	Green	Yellow	Silver	White
Observed	114.0	32.0	22.0	14.0
Calculated 9:3:3:1	102.4	34.1	34.1	11.4
Difference	11.6	—2.1	—12.1	2.6

The P value in this case is 0.11.

The white-podded segregate crossed with silver or yellow would be expected to show a single-factor behavior. When it was crossed with yellow, one yellow-podded F_1 plant was obtained. In the next generation, 38 yellow-podded plants and 9 white-podded ones were produced. The calculated numbers are 35.25 and 11.75, respectively. The difference is 2.75 ± 2.0. White by silver gave one silver-podded F_1 plant. In F_2, 12 plants were silver-podded and 3 were white-podded. The calculated numbers for these figures are 11.25 and 3.75, respectively. The difference is 0.75 ± 1.13.

The single-factor difference between silver and green is shown by a cross of Michigan Robust (green-podded) × Crystal White Wax (silver-podded). The F_1 from this cross consisted of 3 green-podded plants . In F_2 there were 60 plants with green pods and 24 with silver pods. The expected numbers for a three to one ratio are 63 and 21, respectively. The deviation is, therefore, 3 ± 2.68. In the F_3 generation progenies were grown from 10 plants that bore green pods in F_2. Three of the progenies consisted of only green-podded plants and 7 were made up of green- and silver-podded ones. The expected numbers are 3.3 and 6.6, respectively. The deviation is $.3 \pm 1.0$. The 7 heterozygous lines gave a total of 56 plants of which 47 were green-podded. The calculated ratio is 42 and 14, making the deviation 5 ± 2.19. Progenies were also grown from 4 silver-podded F_2 plants. A total of 32 plants were produced, all having silver-colored pods.

SUMMARY AND CONCLUSIONS

Data of three generations from three crosses on the mode of inheritance of stringlessness are presented. Data from two of the crosses can be explained by assuming a single-factor difference between the varieties crossed. F_2 and F_3 results from a third cross can be explained by assuming a two-factor relationship. One dominant factor produced stringlessness but may be inhibited in its action by a second factor. F_2 results from a number of additional crosses indicate the existence of two genetic types of stringless and possibly two types of stringy among the varieties used for the crosses.

Two crosses were studied for three generations to determine the nature of the inheritance of fiber in the side wall of the pod. Owing to difficulties of making classes for this character and to the limited amount of data obtained on it, no definite statement can be made regarding its inheritance.

A cross between the varieties Wardwell and Longfellow indicates that pod width is inherited as a quantitative character and therefore may be associated with a series of multiple factors. The cross was carried through the F_3 generation in one case and through the F_2 in another instance. It is hoped the data at hand may be clarified by growing a number of F_3 populations from the latter cross.

Width of pod is associated with fiber of the side wall of the pod, and the two characters may depend on the same factor or factors.

The inheritance of the pod color of the variety Crystal White Wax was found to give monohybrid results when crossed with green. A limited amount of data on the F_1, F_2, and F_3 of this type of cross was secured. When Crystal White Wax was crossed with a yellow-podded variety the F_2 and F_3 generations gave results which agree with the theoretical numbers for a dihybrid behavior. The double recessive from this cross represents a new pod color so far as known by the author. When crossed with either yellow- or silver-podded plants, the F_2 numbers are approximately in the ratio of three yellow or silver to one double recessive. The color regions for the four types of pods are described with reference to the anatomical structure of the pod.

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