INHERITANCE OF A LEAF VARIEGATION IN BEANS[1]

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INTRODUCTION

In the course of investigations for the development of mosaic resistance in beans (*Phaseolus vulgaris* L.), a leaf variegation was frequently observed in progenies from hybrids in which Corbett Refugee, a selection from Stringless Green Refugee, had been used as one of the parents and also in progenies from hybrids involving other parental stock. The symptoms, which somewhat resembled those caused by a virus, differed from those of the common bean mosaic, and repeated attempts to infect susceptible bean varieties by the usual methods proved that this abnormality was not of a virus nature. It has been demonstrated by a study of these hybrids that this variegation is heritable (*7*).[3]

Other heritable leaf abnormalities and chlorophyll-deficient types in beans have been described by Burkholder and Muller (*1*), Parker (*3*, *4*), Johannsen (*2*), and Tjebbes and Kooiman (*6*).

In 1936 Harrison and Burkholder,[4] and some time later Horsfall, Burkholder, and Reinking,[5] reported in Wisconsin Refugee a disease which appeared to them to be a new virus disease of beans and which they called "one-sided mosaic". The symptoms described for this disease coincide almost exactly with the abnormality described in this paper.

In 1939 Reinking and Withiam (*5*) found that in certain bean plantings in New York as many as 22 to 27 percent of variegated plants were present in Idaho Refugee and in Corbett Refugee, one of the parents of Idaho Refugee. The variegation was not identical in all respects with the condition described in this paper.

The writer has observed variegation in commercial plantings of Wisconsin Refugee and Idaho Refugee beans grown in Colorado and Idaho. Both varieties are of the same parentage. Although the percentage of affected plants was small in most cases, it was not difficult to find abnormal plants.

Two kinds of leaf variegation were encountered in these studies: (1) A type that appeared on the primary leaves and later on the trifoliate leaves if the plant survived, the symptoms on these leaves frequently being more severe than those on the simple leaves; and (2) a type with similar symptoms confined to the trifoliate leaves. This second type of variegation was most common under field conditions.

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[3] Italic numbers in parentheses refer to Literature Cited, p. 126.

[4] HARRISON, A. L., and BURKHOLDER, W. H. CANNING BEAN DISEASES IN NEW YORK IN 1936. U. S. Bur. Plant Indus., Plant Dis. Rptr. 20: 290–291. 1936. [Mimeographed.]

[5] HORSFALL, JAMES G., BURKHOLDER, W. H., and REINKING, O. A. DISEASES OF GREEN REFUGEE BEANS IN NEW YORK IN 1937. U. S. Bur. Plant Indus., Plant Dis. Rptr. 21: 318–319. 1937. [Mimeographed.]

The symptoms of these two types are impossible to differentiate on the trifoliate leaves. Plants with extremely variegated primary leaves usually die. In some cases the symptoms of the type confined to the trifoliate leaves are not noted until the plant has reached considerable size. When these studies were initiated it was assumed that such symptoms noted only on the trifoliate leaves were a delayed expression of the variegation that also appeared on the primary leaves. It has since been determined that the primary-leaf variegation is inherited independently of the type that occurs only on the trifoliate leaves and is governed by different genetic factors. These studies deal only with the inheritance of the leaf variegation that appears on the primary leaves of seedling plants. The data presented are based on results of crosses between Corbett Refugee and several other varieties of beans and of other crosses between variegated and normal green plants.

MATERIAL AND METHODS

The crosses involved in this study are listed in table 1. The hybrids tested in 1937 and 1938 were not made for the purpose of studying the inheritance of variegation; they were made for breeding disease-resistant varieties. The variegation character was not known at that time, and hence the exact nature of all the plants used in the crosses with regard to variegation was not recorded. The symptoms of variegation in Corbett Refugee are in most instances very mild and are readily overlooked. The strain of Corbett Refugee used in the crosses made in 1937 was of this kind, and the variegation was not detected. No record was made of the presence of variegation in the strains of Corbett Refugee used in the crosses tested in 1938, but it may be assumed that no plants showing any unusual abnormality would have been used in hybridization. The Corbett Refugee and the unnamed varieties of which one of the parents was Corbett Refugee, used in the crosses tested in 1939 varied; in some plants the symptoms of variegation were not observed, whereas in others variegation was present in different degrees (see table 1). When selfed, progenies from the parents contributing the factors for variegation produced only a small percentage of variegated plants (see table 2), whereas those from the green parents bred true for the normal green condition.

The crosses (see table 1) were made in the greenhouse at the United States Horticultural Station at Beltsville, Md. The F_1 hybrids were grown under field conditions at Greeley, Colo., and remained free from the common bean mosaic and other virus diseases. Although it is known that natural field hybridization may occur in beans, this was not detected from a study of other characters, and hence it in no way accounted for the deviations which are noted in the segregating generations. The seed of each plant was harvested separately and used later for a study of the F_2 generation. These progenies were grown in the greenhouse, the temperature of which was maintained at from 70° to 80° F., and the plants were classified as soon as the simple leaves were fully developed. Certain F_2 plants were chosen at random within the normal-appearing and variegated classes and grown to maturity to give rise to the F_3 families. The F_3 families were grown both under field conditions and in the greenhouse and were classified in the seedling stage to obtain the F_2 genotypic distribution.

The data were subjected to the χ^2 test for goodness of fit to certain theoretical ratios. This test was applied to F_2 families independently and to F_3 families from F_2 plants.

SYMPTOMS

The symptoms of the leaf variegation described herein always appear on the primary leaves (fig. 1, *G–I*). They may vary considerably, with some leaves practically devoid of the normal green pigmentation (fig. 1, *H*) and with only small islands of light-green tissue interspersed with yellow. In some cases the leaf is almost entirely light-yellowish green in color with small islands of darker green tissue (fig. 1, *H*), whereas in others small islands of dark green may be surrounded by deep-yellow-colored tissue. Occasionally a leaf may show over a small portion of its surface only a slight chlorotic streaking or mottling, which may be readily overlooked. Leaves that are mostly devoid of the green pigment are smaller than those with more chlorophyll, and they usually die (fig. 1, *H*). It is probable that such plants would be overlooked under field conditions unless examinations were made early in their growth.

Symptoms on the trifoliate leaves also are variable. Sectoring is common, with only small portions of the leaf yellow and the remaining portion normal green (fig. 1, *A*, *C*). Not infrequently only a few islands of yellow are noticeable. A very characteristic symptom on plants that have not died in the seedling stage is a variegation of only one side of the leaflet (fig. 1, *A*). Symptoms of this kind were pointed out by Harrison and Burkholder[6] and by Horsfall et al.[7] The unaffected portion grows normally, whereas the chlorotic half does not. Sometimes growth almost completely stops on the chlorotic side of the leaflet; thus, the leaflet becomes decidedly distorted and curls toward the affected portion (fig. 1, *A*). This peculiar symptom suggested the name "one-sided mosaic" to Horsfall et al.[7]

A necrotic streaking or spotting may occur on the petiole and affected parts of the leaflet. The trifoliate leaflets that arise from the necrotic side of the petiole may be affected and only one or sometimes two of the leaflets remain normal (fig. 1, *C*). In like manner, only a portion of the plant may be so affected.

Seriously variegated plants that do not die in the seedling stage sometimes become somewhat rosetted and considerably stunted. The internodes are much shorter than those of normal plants, and adventitious buds commonly arise in the axils of the leaves. Affected plants grown under ideal conditions may sometimes attain maturity and produce a few curled and distorted pods. Plants mildly affected may produce a normal seed crop.

The pods on extremely variegated plants are distorted, frequently curled, and abnormal in shape, size, and color (fig. 1, *D–F*). They sometimes produce seeds that, although appearing normal in most respects, are subnormal in size.

[6] See footnote 4.
[7] See footnote 5.

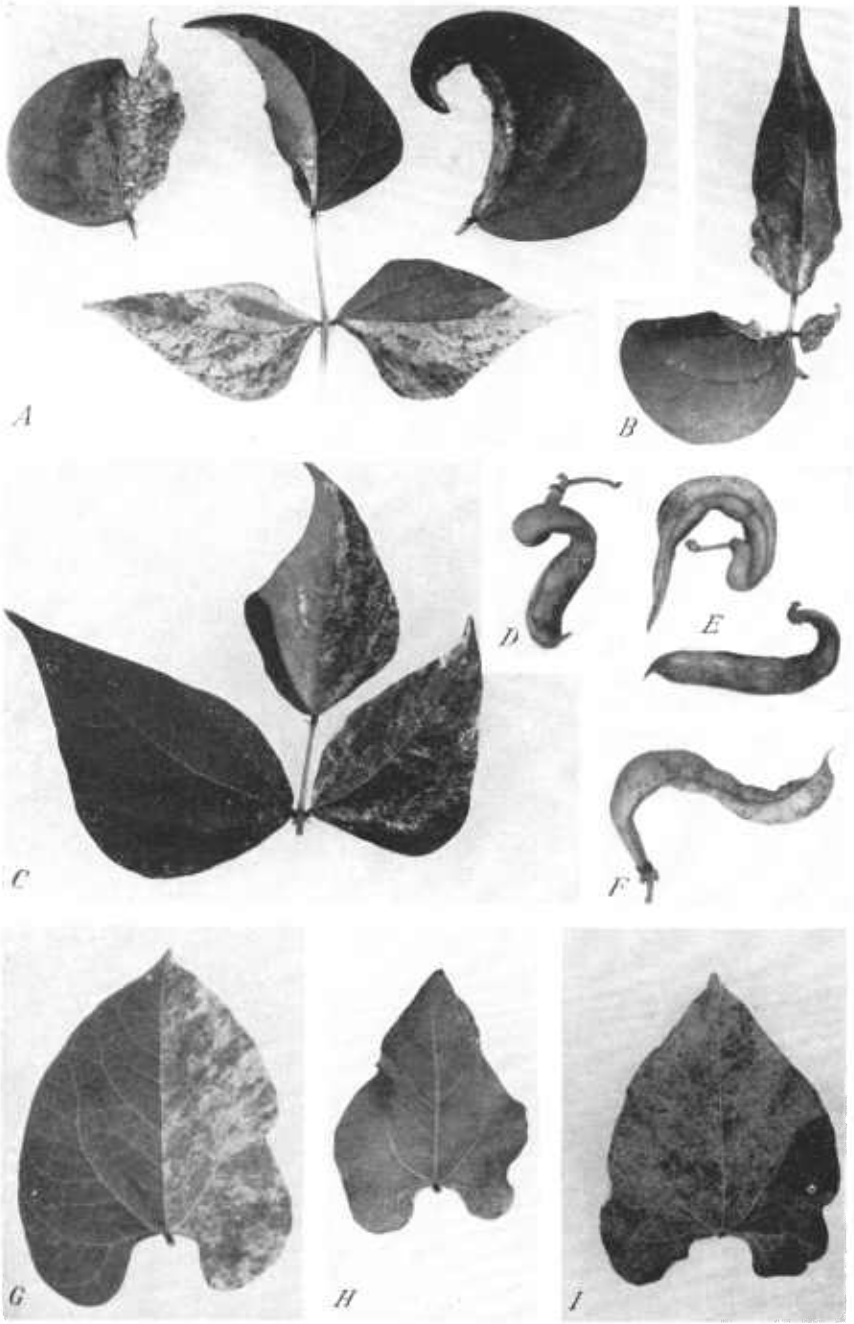

FIGURE 1.—Symptoms of variegation on bean: *A*, F_2 hybrid (Corbett Refugee × Stringless Green Refugee) showing malformation of one side of certain trifoliate leaflets; *B*, trifoliate leaf of another hybrid of same cross with extreme malformation and dwarfing of one leaflet; *C*, trifoliate leaf with only two of the three leaflets showing the variegation; *D–F*, pod malformation due to variegation; *G–I*, variegated primary leaves of Wisconsin Refugee.

EXPERIMENTAL RESULTS

PARENTS

As mentioned previously, variegation in Corbett Refugee was in most instances very mild and was readily overlooked. Although in some instances it was quite evident, it was seldom as severe as that in the segregating generations. In no case did any of the strains of Corbett Refugee or other variegated parents used in the crosses (table 1) breed true for variegation. This fact tends to explain certain results discussed later. The breeding behavior of a number of variegated parents when selfed is recorded in table 2, where it is seen that only a small percentage of their offspring manifested variegation.

TABLE 1.—*F_2 segregations from crosses between Corbett Refugee or other strains or varieties contributing the genes controlling variegation and normal green varieties*

Parentage of crosses	Nature of parent contributing variegation	Year tested	Number of F_2 plants that were—		χ^2 for 15 : 1 ratio [1]
			Green	Variegated	
U. S. No. 1 Refugee × Corbett Refugee	Normal appearing	1937	390	28	0.147
Corbett Refugee × U. S. No. 1 Refugee	do	1937	566	52	4.962
Corbett Refugee × Stringless Green Refugee	do	1937	171	4	4.658
Corbett Refugee × Perry Marrow	Unknown	1938	1,502	85	2.169
Perry Marrow × Corbett Refugee	do	1938	308	19	.108
Geneva Red Kidney × Corbett Refugee	do	1938	1,055	62	.930
Corbett Refugee × Geneva Red Kidney	do	1938	713	29	6.960
Canadian Wonder Bush × Corbett Refugee	do	1938	418	27	.025
Bountiful × Corbett Refugee	Normal appearing	1938	228	23	3.620
Small White × Corbett Refugee	Unknown	1938	332	17	1.127
Unnamed variety (9D)[2] × Blue Lake	Seriously variegated	1939–40	860	39	5.615
Unnamed variety (10A)[2] × Small White	Mildly variegated	1939–40	276	9	4.641
Wisconsin Refugee [3] × Red Kidney	Seriously variegated	1939–40	201	15	.177
Small White × Corbett Refugee	Normal appearing	1939–40	830	47	1.184
Red Kidney × Corbett Refugee	Moderately variegated	1939–40	410	29	.099
Corbett Refugee × Red Kidney	do	1939–40	116	5	.948
Perry Marrow [4] × Great Northern	Unknown	1938	226	15	.001
Cranberry [4] × Pinto	Normal appearing	1938	338	32	3.658
Unnamed variety [3] × No. 780 [5]	Variegated	1938	175	10	.245
Red Kidney [4] × Blue Lake	Normal appearing	1939–40	305	16	.892

[1] 5-percent point=3.841. [2] Corbett Refugee, one of parents. [3] Variegated parent.
[4] Parent contributing genes controlling variegation. [5] Number carried in file of writer.

TABLE 2.—*Breeding behavior of a number of the selfed variegated parents used in the several crosses*

Variegated parent	Strain No. [1]	Number of plants that were—		
		Normal appearing	Variegated	Doubtful
Corbett Refugee	473–B	801	18	0
Do	473–5–B	781	22	25
Do	473–7–B	1,140	12	2
Do	8C	503	3	0
Do	5D	423	4	0
Wisconsin Refugee	6A	429	2	0
Unnamed variety	10A	422	10	0
Do	9D	342	2	0

[1] Numbers carried in files of writer.

F_1 GENERATION

The F_1 plants from reciprocal crosses between Corbett Refugee, as well as certain unnamed varieties, and normal green varieties were all normal green, which indicated that the factor or factors for variegation were recessive to normal green in inheritance. In some cases the Corbett Refugee parent was variegated; in others it was not. The nature of the parents used in the crosses is shown in table 1.

Reciprocal crosses were not made with every hybrid combination, but where they were made and tested they behaved about alike (table 1). This proved that the inheritance was not cytoplasmic.

F_2 GENERATION

From 20 progenies comprising 40 crosses, 9,983 F_2 plants were grown, a part of these being produced in the greenhouse and the others in the field. Fifteen of the twenty progenies showed satisfactory fits to a 15:1 ratio, as is seen in table 1. The other progenies showed significant deviations from this ratio, but only 1 was highly significant. Thirteen of the progenies had deficiencies of variegated recessives.

Although differences in the intensity of the variegation were observed, the plants were not classified as to degree of severity but were recorded only as variegated. Environment, without doubt, influenced the expression of the character, but the wide variation from a mild mottling to extreme malformation could not be reasonably accounted for on the basis of environmental factors alone, since various degrees of variegation occurred simultaneously.

F_3 GENERATION

In the F_3 generation no family of less than 66 plants was used in the analysis.

F_3 progenies were grown from 204 normal green F_2 plants that were derived from 18 original crosses. Of these, 89 progenies bred true for normal green, 61 segregated into 15 green to 1 variegated plant, and 54 segregated into 3 green to 1 variegated plant, which approximated a 7:4:4 ratio, respectively, with a χ^2 value of 1.207.[8] This substantiated the duplicate-factor hypothesis as found in the F_2 generation. Of the 61 progenies that segregated into a 15:1 ratio, 26 were deficient in variegated plants. Among the families that showed a 3:1 segregation, practically every one showed this deficiency.

DOUBLE RECESSIVE CLASS

Several hundred F_2 variegated seedlings were transplanted in order that they might be grown to maturity, but because of the lack of chlorophyll only a small percentage survived. At the beginning of the study the number of transplanted variegated seedlings was not recorded since it was not assumed that they were lethal, and as a result the exact percentage of plants that survived could not be calculated. Later studies, however, showed that from 849 transplanted seedlings derived from 136 F_3 progenies, only 119 plants or 14.0 percent survived and those plants that grew to maturity produced a relatively small number of seeds. In another experiment, 35 variegated seedlings derived from 4 F_2 progenies were transplanted. Only 11 of them survived, and these produced 105 seeds.

It is seen from table 3 that from a total of 139 variegated progenies that survived and were grown in the next generation, only 23 produced all variegated plants. There were only 83 plants produced from these

[8] 5-percent point=5.991.

23 progenies, or an average of less than 4 plants per progeny. It is probable that if the populations had been larger all progenies would have produced some green plants. Among the 37 progenies that produced all green plants, the individual populations were likewise too small to permit definite conclusions. The 79 progenies that produced 230 variegated and 427 green plants likewise had small individual populations. It is probable that if the F_2 variegated plants had produced fairly large progenies all of them would have contained both green and variegated plants.

TABLE 3.—*Breeding behavior of variegated F_2 plants in the F_3 generation*

F_3 segregation	Number of F_3 progenies	Number of F_3 plants that were—	
		Green	Variegated
All green plants	37	124	0
Green and variegated plants	79	427	230
All variegated plants	23	0	83
Total	139	551	313

Since the variegated parents used in the crosses did not breed true for variegation and since the segregating progenies in the F_2 and F_3 generations were deficient in variegated plants, it should not be expected that the double recessive lines would breed true for variegation. As mentioned previously, the progenies that produced all variegated plants were of such small populations that the data appear hardly conclusive. In general the data corroborate those of the parental material as well as those of many of the segregating progenies in that a great number showed deficiencies of variegated plants.

DISCUSSION

The results of crossing Corbett Refugee, as well as several other strains and varieties of beans that carry the factors for variegation, with normal green plants indicate that this condition is heritable and is governed by two major Mendelian factors behaving as recessives. It is evident that the data do not conform in every detail to the hypothesis of duplicate factors.

In spite of the fact that it was shown that the Corbett Refugee parents produced only a relatively few variegated offspring (table 2), it is believed that they were homozygous recessive for both variegated genes. The fact that the expression of variegation was much more pronounced in the F_2 segregates than in Corbett Refugee suggests that one or several inhibiting factors are carried by Corbett Refugee. The belief that such modifying factors exist is further strengthened by the deficiency of recessives in the F_2 generation and in most of the F_3 segregating families and also by the failure of many of the variegated recessives to breed true.

As mentioned earlier, a high percentage of the variegated plants were lethal, and it is assumed that these are the true-breeding recessives. Those that survived and produced seed were probably the ones carrying some of the inhibiting factors, possibly in the heterozygous condition. In the progeny of these plants the variegated

character may again have been suppressed, the action of the inhibitors accounting for the high percentage of green plants in the progeny of the recessive class.

This heritable leaf variegation has not been observed in many bean varieties that are grown commercially, but it has been of importance in the Wisconsin Refugee variety and to a lesser degree in the Idaho Refugee. Both of these varieties have Corbett Refugee as one of the parents. This variety has been used extensively in hybridization because of its immunity to the common bean mosaic virus. In practically all cases where it was used, the progenies exhibited variegation. Although there may be strains of Corbett Refugee that are free from this condition, it should be used as a parent only with caution. This weakness should be recognized and watched for carefully. Since inhibiting factors may suppress the expression of the variegated symptoms, normal-appearing strains may actually carry the factors for this condition and when outcrossed with normal green varieties the variegation would be expressed. If a mosaic-resistant Refugee type bean is desired as a parent, it is suggested that the U. S. No. 5 Refugee had better be used. This variety, although derived from a cross with Corbett Refugee, is free from variegation and is resistant to the common bean mosaic.

SUMMARY

Two types of leaf variegation have been observed in beans, one of which appears both on the primary and the trifoliate leaves and the other only on the trifoliate leaves. The symptoms of the two are similar, but they are inherited differently. The inheritance of the first is described in this paper.

The results obtained when plants of Corbett Refugee and other varieties that carry the factors for variegation were crossed with normal green plants of several varieties are presented. For the most part the data support the two-factor Mendelian hypothesis. The F_1 plants of reciprocal crosses were normal green in appearance, and a ratio of 15 green plants to 1 variegated was obtained in the F_2 generation.

In the F_3 generation from green F_2 plants, a 7:4:4 ratio of all green, 15 green to 1 variegated, and 3 green to 1 variegated was obtained.

The variegated recessive progenies, except in a few instances where the populations were small, did not breed true in the F_3 generation. It is assumed that this lack of true breeding was due to one or several inhibiting factors that suppressed the variegation character. The death of a high percentage of the variegated plants was probably due to the absence of these inhibitors.

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