STUDIES ON THE RESISTANCE OF TWO STRAINS OF NAVY(PEA) BEANS TO VIRUS 15

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by

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INTRODUCTION

Robust bean, a pea bean variety was introduced into Michigan in 1914 by F. A. Spragg. (1)[1]

The Robust variety was developed from a thrifty selection made in 1907. Reddick reported in 1918 that this new variety was resistant to common bean mosaic now called virus 1. (1)

It is probably because of this one characteristic more than any other that the Robust variety was used to plant 85 percent of the Michigan pea bean acerage in 1938. (2)

The Robust variety was used as one of the parents of the Michelite bean, a pea bean that was introduced into Michigan in 1938 by the Michigan Agricultural Experiment Station. (2)

Reports from New York State in 1939 showed Michelite to be susceptible to a new virus. (3) This new virus has been called strain 15, a variant of strain 1 (4), Burkholders strain (5). In the work done here, it has been called strain 15.

The acreage of Michelite beans increased in Michigan until in 1948 when an estimated 400,000 of the 500,000 acres of pea beans were planted to it. Virus 15 was first observed by A. L. Anddrson in Michigan in 1948. (Unpublished)

Because Michelite and Robust varieties are susceptible to virus 15 and all other varieties of pea beans grown in the state are susceptible to virus 1 and virus 15, the spread of virus 15 could do a great amount of damage. Consequently a breeding program was set up by the Michigan Agricultural Experiment Station in cooperation with the Division of fruit,

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[1]Numbers in parentheses refer to literature cited.

vegetable crops and plant diseases of the Bureau of Plant Industry of the U.S.D.A. in 1948.

As a source of resistance to virus 15, a strain of beans number 46-62 selected(by H. M. Munger of Cornell University, Ithaca, New York) from the F-4 generation of a back cross between Michelite X Great Northern 31 recrossed to Michelite was used. This strain has many desirable characteristics, but it has a badly wrinkled seed coat when grown in the field. This fault prevents it from being introduced as a commercial variety. Michelite and selections from several crosses between Robust and Crawford and other varieties were crossed with selection 46-62. It was observed that in the cross between selection number 50021 and 46-62 susceptible and resistant F-1 plants were produced. Immediately, the purity and value of strain 46-62 for virus 15 resistance was questioned.

The present investigation was undertaken to determine the inheritance of virus 15 resistance as found in strain number 46-62.

Ali in 1950 (6) reported on the genetics of resistance to bean virus 1. The results from this indicated that there was dominant resistance present in Corbett Refugee type of beans, and a recessive factor for resistance present in the Robust varieties. His data gave evidence of two pairs of independently inherited gene differences, with dominant and recessive epistasis.

In work reported in 1929(7) from the Experiment Station of the University of Idaho, it was found that at low temperatures the mosaic symptoms of bean virus 1 were reduced in severity, and that there was a much lower percentage of infection of susceptible plants. Susceptible plants were inoculated with virus 1 and placed in chambers at three different levels of temperature. At the end of twenty days, the plants held at the two

higher temperatures (60 and 70 degrees Fahrenheit) showed a considerable amount of mosaic, while those kept at a temperature of 47 degrees Fahrenheit did not show any mosaic symptoms. The plants which had been kept at the low temperatures were then placed in a chamber which had a temperature of 70 degrees Fahrenheit. At the end of two weeks, five of the ten inoculated plants showed mosaic symptoms.

In 1948, Grogan and Walker (5) reported on the effect of temperature on necrosis found on plants which were inoculated with the approach graft method and subjected to different temperatures. They used temperatures of 82, 75, 68, and 60 degrees Fahrenheit. Three strains of viruses that attack beans were used. They were the common mosaic (strain 1) greasy pod, and strain 15. Fourteen days after inoculation many plants kept at 82 and 75 degrees were dead and a few of the plants kept at 68 degrees and inoculated with virus 1 were dead while those inoculated with the greasy pod strain and strain 15 were just beginning to show necrosis at this temperature. In plants kept at 60 degrees, there were a few plants that had been inoculated with virus 1 and with the greasy pod strain which were showing necrosis, but there was no necrosis in the plants inoculated with virus 15. After 28 days practically all of the plants that had showed necrosis earlier were dead in the chambers where the temperatures were maintained at 82, 75, and 68 degrees. Plants which were inoculated with virus 1 and greasy pod strain and maintained at 60 degrees were showing a high percentage of necrosis but no plants were dead. Plants which had been inoculated with virus 15 and kept at 60 degrees showed a slight mottle on one variety while on the second variety there were no symptoms. When the plants which showed no symptoms were moved to a house with a temperature of 82 degrees, they ddveloped necrosis after five days.

MATERIALS AND METHODS

Two strains of navy pea beans were used in this investigation. One strain, which was susceptible to virus 15, was selection number 50021 from the cross between the Robust and the Crawford pea beans. The other strain was selection number 46-62 made by H. M. Munger of Cornell University, Ithaca,New York. This strain originated from a cross between the Michelite pea bean and Great Northern 31. Michelite is susceptible to virus 15 and has the Robust variety as one of its parents, Great Northern 31 is a Great Northern type of bean and is resistant to virus 15. The F-1 plants were back crossed to Michelite for three generations with the resistant plants being selected. Strain 46-62 is resistant to virus 15 and is nine days earlier than the Michelite, but it has a badly wrinkled seed coat when grown in the field. This fault prevents its introduction as a commercial variety.

The strain of virus 15 which was used was one that was obtained from W. J. Zaumeyer of the U.S.D.A. It was carried on susceptible plants in the green house and had been used in previous work in this program.

All the crossing and the testing for disease resistance was done in the greenhouse. The temperatures in the green house at the time of inoculation varied to a considerable extent with the outside temperatures. The temperature was fairly high when the plants were inoculated in the early fall while the temperatures were fairly low when the plants were inoculated in early January. The temperatures were observed to be down to about 50 degrees Fahrenheit in the mornings during January. However there were no daily temperature recordings made. The greenhouse was kept almost free of insects by frequent fumigations.

Cross pollination was carried out in two different ways. In method one the anthers were removed by the use of forceps from the flower that was to be used as the female parent. Then a stigma was taken from a flower that had already pollinated and which was to be used as the male parent. (2) This stigma was used to pollinate the stigma of the female flower. In method two the emasculation of the flower to be used as the female parent was done through the use of suction. The vacuum was supplied by an ordinary tank type vacuum sweeper from which a rubber tube was extended. This tube had a pointed glass tip fitted in the end. The tip was used to draw off the anthers one at a time. Pollination was done in the same manner as in method one. The results from this method were as good as from using the forceps and it was faster.

The plants were inoculated with the virus in two ways. In method one known as the rub method (2) the carborundum was spread on the primary leaf and then the leaf was rubbed with a pad of cheese cloth that had been saturated in a suspension of virus 15. The suspension of virus 15 was made by extracting the juice from diseased leaves. This juice was diluted with distilled water at a ratio of five parts water to one part juice. Method two consisted of spraying the primary leaves with the suspension of the virus into which carborundum had been placed. The juice was diluted with nine parts of water to one part of juice. The plants were all inoculated when they reached the primary leave stage.

DISCUSSION

Both susceptible and resistant plants were found in the F-1 generation of crosses between strains 50021 and 46-62. Therefore, work was started to determine if there were any differences in reaction of different plant selections of strain 46-62. Selections of strain 46-62 reacted differently when inoculated with virus 15. In inoculating a second generation of these same selections, they did not always react in the same manner. This indicated that the differences might be due to invironmental conditions rather than a difference in genetic constitution. Results obtained when strains of 46-62 used as a female were crossed with strain 50021 are shown in table 1. All of the selections gave similar results in the F-1 and F-2 generations regardless of the reaction of the parent selection. This indicates that strain 46-62 is homozygous for resistance and the difference in reaction of individual plants to inoculation with virus 15 is due to environmental factors.

All of the F-1 generation of plants was susceptible to virus 15 when they were inoculated in the fall.(Table 2) In the crosses where strain 50021 was used as the female, the results were practically the same.(Table 3) This would indicate that resistance to virus 15 was governed by a recessive factor or factors. The F-1 generation of plants which was inoculated in the winter segregated into a ratio of about five resistant plants to one susceptible.(Table 4) In the crosses where strain 50021 was used as the female, the F-1 plants segregated into a a ratio of 23 resistant plants to one susceptible(table 5), indicating that resistance to virus 15 was governed by a dominant factor or factors.

The F-2 generation which was inoculated in the winter was from the F-1 generation that had been inoculated in the fall. This F-2 seg-

Table 1

Shows the reactions of the F-1 and F-2 generation to virus 15 from crosses between susceptible strain 50021 and different selections of resistant strain 46-62 as the female.

Section 1 selections showing no reaction to inoculation.

(a) F-1 plants inoculated in fall and F-2 plants inoculated in winter.

Selection number	F-1 generation		F-2 generation F-1 resistant		F-2 generation F-1 susceptible	
	Res.	Sus.	Res.	Sus.	Res.	Sus.
02	0	2			28	12
10	0	3			18	11
Total	0	5			46	23

(b) F-1 plants inoculated in winter F-2 plants inoculated in spring.

02	1	0	3	9		
10	3	5	8	13	9	26
total	4	5	11	22	9	26

Section 2 selections showing vein browning on the lower leaves when inoculated.

(a) F-1 plants inoculated in winter F-2 plants inoculated in the spring.

05	1	1	1	3	2	9
06	11	1	21	36	2	10
14	0	1	no plants			
19	5	2	16	46	2	9
total	17	5	38	85	6	28

Section 3 selections showing necrotic flecking of stem and petiole when inoculated.

(a) F-1 plants inoculated in fall F-2 plants inoculated in winter.

08	0	8			90	24
09	0	4			62	18
total	0	12			152	42

(b) F-1 plants inoculated in winter F-2 plants inoculated in spring.

03	5	0	13	36		
04	3	0	9	25		
08	1	1	2	10	0	5
09	19	2	60	140	4	8
11	7	0	23	61		
12	4	1	13	36	no plants	
15	3	0	12	24		
17	3	1	13	25		
total	45	5	145	357	4	13

Table 1 (Continued)

Section 4 selections showing lower leaf necrosis when inoculated.
(a) F-1 plants inoculated in winter F-2 plants inoculated in spring.

Selection number	F-1 generation		F-2 generation F-1 resistant		F-2 generation F-1 susceptible	
	Res.	Sus.	Res.	Sus.	Res.	Sus.
01	10	1	16	87	3	8
13	10	1	25	79	no plants	
16	7	1	13	35	no plants	
total	27	3	54	201	3	8

Table 2

Shows the reactions of the F-1 and F-2 generations to virus 15 from crosses between susceptible strain 50021 and different selections of resistant strain 46-62 as the female when the inoculation of the F-1 was made in the fall and the F-2 was inoculated in winter.

Selection Number	F-1 generation Res.	F-1 generation Sus.	F-2 generation F-1 plants sus. Res.	F-2 generation F-1 plants sus. Sus.
02	0	2	28	12
08	0	8	90	24
09	0	4	62	18
10	0	3	18	11
Total	0	17	198	65

F-2 segregation. Chi square(3-1) 0.114 P. range 0.70-0.80

Table 3

Shows the reactions of the F-1 and F-2 generations to virus 15 from crosses between susceptible strain 50021 and different selections of resistant strain 46-62 as the male when the inoculation of the F-1 was made in the fall and F-2 was inoculated in winter. No selection numbers used.

	F-1 generation Res.	F-1 generation Sus.	F-2 generation Res.	F-2 generation Sus.
	1	8	78	34
	0	5	43	9
	1	7	50	63
Total	2	20	171	106

regated into a ratio of approximately three resistant plants to one susceptible.(Table 2) In the F-2 generation where strain 50021 was used as the female in the F-1, the ratio was less than two resistant plants to one susceptible.(Table 3) Again this would indicate that the resistance to virus 15 was dominant and due to one pair of factors. The F-2 generation which was inoculated in the spring was from the F-1 plants that were inoculated in the winter. This F-2 population segregated into a ratio of approximately one resistant plant to three susceptible.(Table 4) When strain 50021 was used as the female in the F-1 generation, the F-2 population segregated into a ratio of approximately one resistant plant to three susceptible.(Table 5) These results indicate again that resistance to virus 15 was recessive and due to a one factor difference.

The back cross populations are small but they verify the results obtained with the F-1 and F-2 populations. The BC-1 generation in which the F-1 plants were used as the female and strain 50021 as the male was inoculated in the winter. The BC-1 population segregated into a ratio of approximately one resistant plant to one susceptible.(Table 6) This would indicate a dominant type of resistance to virus 15 and due to one pair of factors.

The BC-1 generation in which strain 50021 was used as the female and F-1 plants as the male was inoculated in the apring. This generation was almost all susceptible to virus 15.(Table 7) This would indicate a recessive type of resistance to virus 15.

The difference in results in which dominant and recessive resistance is indicated is explained by incomplete penetrance of a single recessive factor pair. This is when the heterozygote is influenced by environmental conditions. That is it may be resistant or susceptible

Table 4

Shows the reactions of the F-1 and F-2 generations to virus 15 from crosses between susceptible strain 50021 and different selections of resistant strain 46-62 as the female when the inoculation of the F-1 plants was made in winter and the F-2 plants were inoculated in spring.

Selection number	F-1 generation		F-2 generation F-1 resistant		F-2 generation F-1 susceptible	
	Res.	Sus.	Res.	Sus.	Res.	Sus.
01	10	1	16	87	3	8
02	1	0	3	9		
03	5	0	13	36		
04	3	0	9	25		
05	1	1	1	3	2	9
06	11	1	21	36	2	10
08	1	1	2	10	0	5
09	19	2	60	140	4	8
10	3	5	8	13	9	26
11	7	0	23	61		
12	4	1	13	36	no plants	
13	10	1	25	79	no plants	
15	6	0	12	24		
16	7	1	13	35	no plants	
17	3	1	13	25	2	7
19	5	2	16	46	2	9
total	89	17	248	665	24	77

F-2 segregation Chi square(3:1) 2.57 P. range 0.10-0.20

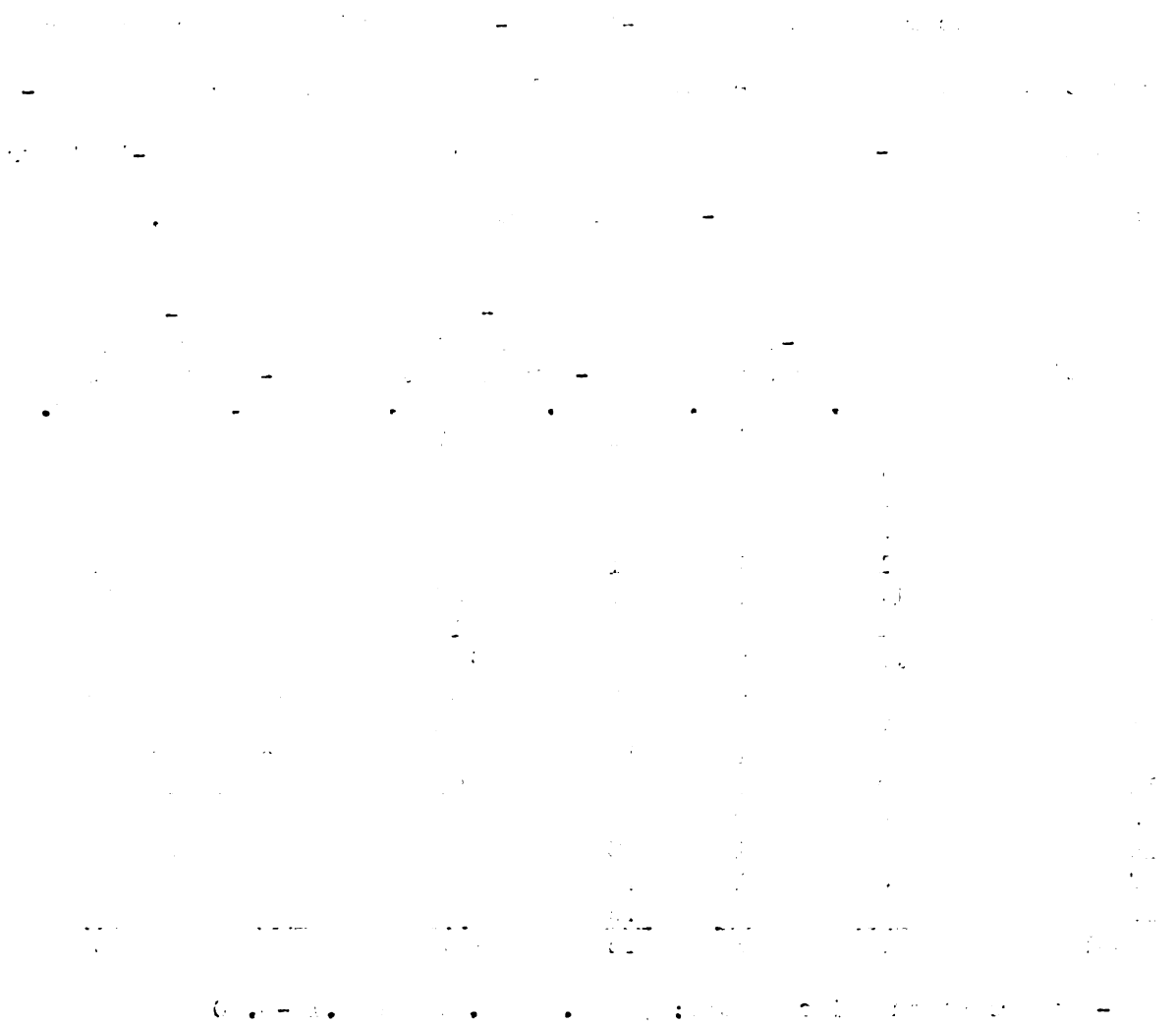

Table 5

Shows the reactions of the F-1 and F-2 generations to virus 15 from crosses between susceptible strain 50021 and defferent selections of resistant strain 46-62 as the male when the inoculation of the F-1 was made in the winter and of the F-2 plants in the spring.

Selection number of male	F-1 generation Res.	F-1 generation Sus.	F-2 generation F-1 resistant Res.	F-2 generation F-1 resistant Sus	F-2 generation F-1 Susceptible Res.	F-2 generation F-1 Susceptible Sus.
01	7	0	20	65		
04	5	0	14	34		
06	7	1	29	57	no plants	
12	9	0	29	78		
14	6	0	18	53		
15	8	0	25	73		
17	4	1	12	37	no plants	
total	46	22	147	397		

Table 6

Shows the reaction of the BC-1 generation to virus 15 when susceptible strain 50021 was used as the male and the F-1 plants were used as the female. No selection numbers were used. Inoculations were made in the winter.

	BC-1 generation	
	Res.	Sus.
	0	1
	1	0
	0	3
	5	0
	1	1
Total	7	5

depending on the environment. When the temperatures are cool, the heterozygote is resistant to virus 15, and when the temperatures are hot, the heterozygote is susceptible. So the results given would indicate that resistance to virus 15 in strain 46-62 is due to a recessive single factor pair of genes. This is shown by the ratio of one resistant to three susceptible plants in the F-2 generation, and by the all susceptible BC-1 generation, both of which were inoculated in the spring. Also by the F-1 generation which was susceptible when inoculated in the fall. The ratio of three resistant plants to one susceptible and the ratio of one resistant plant to one susceptible in the BC-1 generation both of which were inoculated in the winter can be explained by the cool temperatures causing the heterozygote to be resistant.

These data establish the fact that resistance to virus 15 in strain 46-62 is governed by a single recessive factor, that expresses itself at low temperatures as three resistant plants to one susceptible and at high temperatures as one resistant plant to three susceptible.

Table 7

Shows the reactions of the BC-1 generation to virus 15 when susceptible strain 50021 was used as the female and the F-1 plants were used as the male. Inoculations were made in the spring. The number of the selection of resistant strain 46-62 used in making the cross for the F-1 generation is given under the selection number.

Selection number	BC-1 generation Res.	Sus.
01	0	5
02	1	4
03	2	6
04	0	9
05	0	4
06	1	2
08	0	10
09	0	10
10	1	5
11	0	10
12	0	6
14	0	5
17	5	3
total	8	79

SUMMARY

The Michelite variety of pea beans, which is used to plant most of the pea bean acreage in Michigan, is susceptible to virus 15 a new virus disease that infects beans. Therefore, a breeding program was started to incorporate resistance to virus 15 into the Michelite variety. As a source of resistance strain 46-62, from Cornell University, Ithaca, New York, was used. When this strain was crossed with several other strains of susceptible beans, both susceptible and resistant F-1 plants were produced. Therefore, this investigation to determine the genetic inheritance to resistance to virus 15 as found in strain 46-62 was carried out. Resistant strain 46-62 was crossed with susceptible strain 50021 to study the genetic inheritance.

The F-1 plants produced from this cross were all susceptible when they were inoculated in the fall, indicating that resistance was governed by a recessive factor or factors. Another F-1 generation was inoculated in the winter and these plants segregated into a ratio of approximately five resistant plants to one susceptible, indicating a dominant type of resistance.

The F-2 generation of the F-1 plants which were inoculated in the fall segregated into a ratio of approximately three resistant plants to one susceptible, indicating that resistance was governed by a dominant single factor pair. The F-2 generation of the F-1 plants which were inoculated in the winter segregated into a ratio of approximately one resistant plant to three susceptible, indicating that resistance was governed by a recessive single factor pair.

The BC-1 generations which were inoculated in the winter and in the spring were small but they verify the other findings by indicating dominant and recessive factors in the winter and spring respectively.

This variance in results can be explained by the effect of temperature on the heterozygote. The recessive gene exhibits incomplete penetrance. When the temperatures are low the heterozygote is resistant to virus 15, but when the temperatures are high the heterozygote is susceptible to virus 15.

These data establish the fact that resistance to virus 15 in strain 46-62 is governed by a single factor pair of genes. Resistance is expressed in the heterozygote as a recessive gene when the temperatures are high and as a dominant gene when the temperatures are low.

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