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EXAMINATION OF THE INHERITANCE OF RESISTANCE TO *PHYTOPHTHORA MEGASPERMA* VAR. *SOJAE* IN TWO SOYBEAN PLANT INTRODUCTIONS

by

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Introduction

Phytophthora megasperma (Drechs.) var. sojae (Hildeb.), a pathogen of soybeans (Glycine max (L.) Merr.) has been reported to have nine pathogenic races (2,7,8,9,10). Phytophthora (race 1) was first reported in Ohio in 1955 (11) and race 2 was reported in 1965 (8). From 1972 to the present, seven additional races have been found (2,7,9,10).

Phytophthora is a soil-borne pathogen, which produces motile zoospores in soil water. It overwinters as oospores on debris left in the field. Due to the relatively short distance motility of Phytophthora, races tend to be localized. However, Phytophthora is found in most of the major soybean producing areas of the United States and Canada. Race discovery has been dispersed throughout these areas. Races were reported from Ohio (9,11), Mississippi (8), Kansas (10), Ontario (2), and Indiana (7). At the present, races 1,3,4,5,6 and 9 have been found in Kansas.

Bernard et al. (1) reported that resistance to Phytophthora was controlled by an allele at a single locus, Rps. Lam-Sanchez et al. (6) concluded Rps was widely distributed in the soybean gene pool. After the discovery of race 2, host genes differentiating race 1 and 2 were found to be an allelomorphic series at the locus previously found to control resistance to race 1 (3). Rps confers resistance to both races, rps^2 confers resistance to race 1, and rps produces a susceptible reaction to both races. Rps is dominant to rps^2 and both Rps and rps^2 are dominant to rps. Kilen et al. (5) discovered that by using a medium inoculation technique, a second major gene for resistance to race 1 could be found. Kilen suggested the symbols, Rps_1, rps_1^2, rps_1 and Rps_2, rps_2, be used to distinguish the two loci. Kilen has found four additional loci controlling resistance to race 1 (personal communication). Kilen also indicates two loci involved in resistance to race 4 in 'Tracy' (personal communication).

This study was undertaken to analyze the genetics of host resistance in two plant introductions. Races 1,3 and 4 were used to analyze for the number of host loci controlling resistance to each race and the interrelationships between races to loci controlling resistance.

Materials and Methods

Plant materials used in this study were concurrently being used to introduce Phytophthora resistance into the breeding program at Kansas State. Therefore, the plant introductions, which are not agronomically desirable, were crossed to lines with good agronomic potential. K16-12-71-270 is a line selected from the cross, 'Wayne' x (('Harosoy' x ('Lincoln' x 'Ogden'))^7 x 'Mukden') and is known to carry, Rps_1, the Mukden source of resistance to races 1 and 2. K69-8-72-510 and K1016 are selections from the cross, 'Williams' x 'Columbus' and are known to carry rps_1. K16-12-71-270, K69-8-72-510, K1016 and Williams are agronomically desirable. Plant introductions were selected because they are resistant to races 1,2,3 and 4 (Table 1).

The cross, K74-20R, allows comparison of the genes involved in host resistance to race 1, between the Mukden source and PI54606-1 (Table 1). Crosses, K74-37H and K74-43H, allow the genes for resistance involved in PI54606-1 to be analyzed. These two crosses should have homogeneous segregation ratios, as K69-12-71-270 and K1016 are closely related selections. Analysis of the segregation ratios of K74-49H, K74-37H, and K74-43H will give a comparison of the genes for

Table 1. Reaction of the parents used in crosses to races 1,2,3 and 4 of *Phytophthora megasperma* var. *sojae*.

Parent	Race 1	2	3	4
PI54606-1	R	R	R	R
FC31630	R	R	R	R
K16-12-71-270	R	R	S	S
K69-8-72-510	S	S	S	S
K1016	S	S	S	S
Williams	S	S	S	S

R=resistant
S=susceptible

Table 2. Parentage of crosses used in the study.

Cross	Parents
K74-20R	K16-12-71-270 x PI54606-1
K74-37H	K69-8-72-510 x PI54606-1
K74-43H	K1016 x PI54606-1
K74-49H	Williams x FC31630

resistance involved in FC31630 with those involved in PI54606-1.

Crosses were made in the field in the summer of 1974. F_1 plants produced seed in the summer of 1975. The F_2 seed were planted in the greenhouse during the winter of 1975.

F_2 seedlings were inoculated using a hypocotyl inoculation technique as described by Kaufmann and Gerdemann (9). Seedlings, 10 to 14 days old, were inoculated by making an incision in the hypocotyl, placing agar containing growing mycelium from a particular race of Phytophthora megasperma var. sojae in the wound, and sealing the wound with petrolatum.

F_2 seedlings found resistant to Phytophthora were transplanted to the field to produce seed. Seed from single F_2 plants were collected and catagorized by the cross and race used in the F_2 screening. In the winter of 1976, ten seeds from each F_2 plant were planted per pot in the greenhouse. Seedlings from plants found resistant in the F_2 screening to either race 1 or 4, were inoculated with races 1 and 4 in the F3 screening. Two pots from each of the F_2 plants were inoculated with each race. Seedlings from plants found resistant to race 3 in the F_2 were inoculated with races 1,3 and 4. The same inoculation technique was used in the F_3 as in the F_2. Williams, 'Cutler 71', 'Mack', and Tracy were used as check cultivars (Table 3).

The race 1 culture used in the majority of this study became contaminated with race 4 during the F_3 screening. A second race 1 culture was used for the remainder of the study. Both cultures were originally isolated from plants at the Southeast Kansas Branch Experiment Station during the same year. The race 3 culture used was isolated from plants found near Omaha, Nebraska. The race 4 culture was isolated from 'Clark 63' growing in central Kansas.

Chi-square analysis was used to compare the observed ratios with the expected ratios for various models. Homogeneity tests were used to compare the ratios found in the various crosses, where logically applicable.

Table 3. Reaction of cultivars used as checks to races 1,3 and 4 of *Phytophthora megasperma* var. *sojae*

	Race		
Cultivar	1	3	4
Williams	S	S	S
Cutler 71	R	S	S
Mack	R	R	S
Tracy	R	R	R

R=resistant
S=susceptible

Results and Discussion

K74-20R, K74-37H and K74-43H have the plant introduction, PI54606-1, in common as a resistant parent to races 1,3 and 4. K74-20R receives the Rps_1 allele from K16-12-71-270. F_2 data for K74-20R screened with race 1 fits a 15:1 ratio, indicating a two loci interaction controlling resistance, where one dominant allele at either locus is needed for resistance (Table 4). In K74-37H and K74-43H, the F_2 ratios when screened with race 1 fit a 3:1 ratio, indicating one locus involved in resistance. This model follows logically from the genetic back-

Table 4. Chi-square, expected and observed ratios of F_2 populations for crosses inoculated with Phytophthora megasperma var. sojae, races 1,3 and 4.

Race	Cross	Exp. ratio Res.	Exp. ratio Sus.	Obs. ratio Res.	Obs. ratio Sus.	Chi-Square
1	K74-20R	15	1	93	4	0.71
	K74-37H	3	1	56	10	2.97
	K74-43H	3	1	66	16	1.06
	K74-49H	15	1	79	6	0.21
3	K74-20R	33	17	54	28	0.00
	K74-43H	33	17	47	32	1.41
	K74-49H	3	1	86	17	4.17
4	K74-20R	33	17	55	30	0.05
	K74-37H	33	17	65	34	0.00
	K74-43H	33	17	45	39	5.27

The 33:17 ratio is expected for two loci linked at a distance of 20 map-units when a dominant allele is needed at both loci for resistance.

ground of the crosses. One additional locus, different from the $\underline{Rps_1}$ locus, controlling resistance to race 1 is found in PI54606-1.

Screening with race 3 or 4 in the F_2 produces homogeneous ratios in K74-20R, K74-37H, and K74-43H (Table 5). The ratios do not fit a 3:1 or a 9:7 ratio but fall between these two expected ratios. This suggests a model where two loci are linked and dominant alleles are needed at both loci for resistance. The data obtained in the experiment are not sufficient to estimate the degree of linkage. A 20 map-unit distance between the loci was used to calculate the Chi-square values and in the homogeneity tests (Tables 4 and 5).

By examining the progeny of single F_2 plants, the F_2 plants can be classified as homozygous resistant, heterozygous or homozygous susceptible. In K74-20R, the F_2 plants were screened with race 4, the resistant plants grown to seed and then the progeny screened with race 1. None of the F_2 plants found resistant to

Table 5. Homogeneity test of the ratios of F_2 plants from crosses involving PI54606-1 when screened with races 3 or 4 of *Phytophthora megasperma* var. *sojae*.

Cross	Race	F_2 plant ratios Res.	Sus.	Chi-square
K74-20R	3	54	28	0.00
	4	55	30	0.05
K74-37H	4	65	34	0.00
K74-43H	3	47	32	1.41
	4	45	39	5.27
		266	163	X_t^2 =6.73
		X_d^2 =3.05	X_h^2 =3.68	

Chi-square values are calculated using 33:17 as the expected ratio.
X_t^2 is the total of the Chi-square values for each class with 5 df.
X_d^2 is the Chi-square value of the total of the classes with 1 df.
X_h^2 is the difference between X_t^2 and X_d^2 and tests whether the classes fit the hypothesis and are segregating in the same ratio. Df_h=4.

race 4 were found susceptible to race 1 (Table 6). This indicates a relationship between the genes controlling resistance to race 1 and 4. In the opposite situation, where plants were screened with race 1 in the F_2 and then the progeny of resistant plants with race 4, F_2 plants susceptible to race 4 were found (Table 6). Thus, screening K74-20R with race 4 removes plants susceptible to race 1, but screening with race 1 does not remove all plants susceptible to race 4. In K74-20R and K74-43H, when plants were screened with race 3 in the F_2 and race 1 in the F_3, no F_2 plants susceptible to race 1 were found (Table 6). This along with the similarity of F_2 ratios leads to the conclusion that resistance to race 3 and 4 are controlled by the same two loci. The interaction between race 1 and races 3 and 4 best fit a model where the locus in PI54606-1 involved in race 1 resistance is one of the two loci involved in races 3 and 4 resistance. Thus, two new loci are proposed. $\underline{Rps_3}$ and $\underline{Rps_4}$ are closely linked. The alleles, $\underline{Rps_3}$ and $\underline{Rps_4}$ are dominant to $\underline{rps_3}$ and $\underline{rps_4}$, rspectively. In K74- 20R, the $\underline{Rps_1}$ and $\underline{Rps_3}$ loci are segregating in the F_2, when screened with race 1, to give a 15:1 ratio. One dominant allele, at either locus, is needed for resistance. The Rps_3 locus segregates in K74-37H and K74-43H to give a 3:1 ratio when inoculated with race 1 in the F_2. $\underline{Rps_3}$ and $\underline{Rps_4}$ are the loci controlling resistance to race 3 and 4 in all three crosses. A dominant allele is needed at both loci in order for resistance to races 3 and 4 to occur.

The F_3 data does not support this model, totally (Tables 6 and 7). In K74-37H and K74-43H, the ratio of F_2 plants found from the segregation of F_2 progeny for plants screened with race 1 in the F_2 and race 4 in the F_3 contain no plants susceptible to race 4. When the same crosses are screened with race 4 in the F_2 and race 1 in the F_3, F_2 plants susceptible to race 1 are found (Table 6). This would not be expected by our model. The F_3 plant ratios are all skewed toward the resistant plants, except in K74-37H and K74-43H when race 1 is used in the F_3

Table 6. Chi-square, observed and expected ratios of F_2 plants found by screening the F_2 progeny with races 1,3 or 4 of Phytophthora megasperma var. sojae.

Cross	Race in F_2	Race in F_3	Obs. ratio Res	Seg	Sus	Exp. ratio Res	Seg	Sus	X^2-value
K74-20R	1	1	56	32	0	41	47	0	10.28
	4	1	86	23	0	52	57	0	42.51
	3	1	46	15	0	22	39	0	40.95
	4	4	45	61	0	26	80	0	18.40
	1	4	34	46	8	19	59	10	15.11
	3	4	22	38	0	15	45	0	4.35
	3	3	18	42	0	15	45	0	0.80
K74-37H	1	1	14	40	0	18	36	0	1.33
	4	1	8	24	8	15	25	0	14.16
	4	4	19	21	0	10	30	0	10.80
	1	4	18	35	0	11	36	6	10.48
K74-43H	1	1	8	45	0	18	35	0	8.41
	4	1	8	37	10	20	35	0	17.31
	3	1	19	12	0	11	20	0	9.02
	4	4	24	33	0	16	41	0	5.56
	1	4	21	50	0	15	47	9	11.59
	3	4	19	12	0	8	23	0	20.39
	3	3	14	17	0	8	23	0	6.07
K74-49H	1	1	17	39	0	19	37	0	0.33
	3	1	42	14	0	20	36	0	37.64
	1	4	15	41	0	12	37	7	8.18
	3	4	25	31	0	14	42	0	11.52
	3	3	10	46	0	14	42	0	1.24

The expected ratios used in this table are those expected for a model involving three loci, two of which are linked at 20 map-units. The linked loci are involved in resistance to races 3 and 4, a dominant allele needed at both loci for resistance. Race 1 is controlled by the third locus and one of the linked loci, with one dominant allele needed at either locus for resistance, in K74-20R and by the same linked locus in K74-37H K74-43H and K74-49H.

Table 7. Chi-square, expected and observed ratios of F_3 plants screened with races 1,3 and 4 of *Phytophthora megasperma* var. *sojae* in the F_2 and F_3 generations.

Cross	Race in F_2	Race in F_3	Obs. ratio Res.	Obs. ratio Sus.	Exp. ratio Res.	Exp. ratio Sus.	X^2-value
K74-20R	1	1	1431	78	1374	125	19.28
	4	1	1800	40	1730	110	47.38
	3	1	942	41	924	59	6.99
	4	4	1534	221	1330	425	129.21
	1	4	1133	369	801	701	294.81
	3	4	817	142	727	232	46.06
	3	3	776	164	712	228	23.72
K74-37H	1	1	671	155	688	138	2.51
	4	1	404	200	508	96	133.96
	4	4	566	87	495	158	42.09
	1	4	817	139	637	319	152.43
K74-43H	1	1	669	221	742	148	43.19
	4	1	519	261	653	124	180.10
	3	1	400	26	358	68	30.87
	4	4	717	93	614	196	71.40
	1	4	997	188	790	395	162.72
	3	4	381	49	329	104	37.03
	3	3	356	98	344	110	1.73
K74-49H	1	1	769	128	748	149	3.55
	3	1	830	25	719	136	107.73
	1	4	794	118	608	304	170.70
	3	4	825	72	680	217	128.61
	3	3	700	169	689	180	0.85

The expected ratios used in this table are those expected for a model involving three loci, two of which are linked at 20 map-units. The two linked loci are involved in resistance to races 3 and 4, a dominant allele needed at both loci for resistance. Race 1 is controlled by the third locus and one of the linked loci, with one dominant allele needed at either locus for resistance, in K74-20R and by the same linked locus in K74-37H, K74-43H and K74-49H.

screenings (Table 7). The F_3 ratios found in all three crosses involving PI54606-1 for races 3 and 4 fall between that expected for one locus segregating and that for two loci segregating, where one dominant allele is needed at either locus for resistance. Thus, no model we can propose will fit both the F_2 and F_3 plant ratios.

Homogeneity tests (Tables 8 and 11) were run on groups of screening types, i.e. those plants in all crosses screened with race 3 in both generations. These tests indicate that K74-20R, K74-43H and K74-37H (in the group screened with race 4 in both generations) are homogeneous within groups, for groups screened with race 3 in both generations (Table 7), for groups with race 4 in both generations (Table 6) and for groups screened with race 3 in the F_2 and race 4 in the F_3 (Table 10). This lends reliability to the F_3 data. However, from our F_2 model, we would expect the three screening groups to be homogeneous with each other. The group screened with race 3 in both generations is not homogeneous with the other two groups (Table 11).

FC31630 and PI54606-1 must be considered to have different genes for resistance. K74-49H, which has FC31630 as the resistant parent, fits a 15:1 ratio in the F_2 when screened with race 1 (Table 4). This would indicate two loci controlling resistance to race 1 in FC31630, where PI54606-1 has one locus. K74-49H when screened with race 3 in the F_2 produces a ratio which falls between a 3:1 ratio and a 15:1 ratio but fits neither (Table 4). This is not consistent with the PI54606-1 crosses. F_3 ratios for K74-49H are not homogeneous with the other crosses for plants screened with race 3 in the F_2 and race 4 in the F_3 (Table 10).

K74-49H appears to have two loci controlling resistance to race 1. The inheritance of resistance in K74-49H to race 3 is not totally clear. There is an interaction between loci controlling resistance to races 1,3 and 4, however, not enough information is available for interpretation.

The locus controlling resistance to race 1 in PI54606-1 is different from the Mukden source of resistance and can be considered a new source of resistance.

Table 8. Homogeneity test of ratios of plants screened with race 4 in both the F_2 and F_3

Cross	F_3 plant ratios Res.	Sus.	Chi-square
K74-20R	1534	221	129.69
K74-37H	566	87	42.39
K74-43H	717	93	71.82
$Total_1$	2817	401	X_t^2 =243.90

X_d^2 =243.07 X_h^2 =0.83

Chi-square values are calculated using 25:8 as the expected ratio.
X_t^2 is the total of the Chi-square values for each class with 3 df.
X_d^2 is the Chi-square value of the total of the classes with 1 df.
X_h^2 is the difference between X_t^2 and X_d^2 and tests whether the classes fit the hypothesis and are segregating in the same ratio. Df_h=2.

Table 9. Homogeneity test of ratios of plants screened with race 3 in both the F_2 and F_3

Cross	F_3 plant ratios Res.	Sus.	Chi-square
K74-20R	776	164	23.64
K74-43H	356	98	1.74
$Total_2$	1132	262	X_t^2 =25.38
	X_d^2 =22.53		X_h^2 =2.85
$Total_2$	1132	262	25.38
K74-49H	700	169	2.73
	1832	431	28.11
	X_d^2 =33.28		X_h^2 =5.17

Chi-square values are calculated using 25:8 as the expected ratio.
X_t^2 is the total of the Chi-square values for each class with 2df for $total_2$ and 3df for overall total.
X_d^2 is the Chi-square value of the total of the classes with 1df.
X_h^2 is the difference between X_t^2 and X_d^2 and tests whether the classes fit the hypothesis and are segregating in the same ratio. Df_h for $total_2$ =1. Df_h for overall total =2.

Table 10. Homogeneity test of ratios of plants screened with race 3 in F_2 and race 4 in F_3

Cross	F_3 plant ratios Res.	Sus.	Chi-square
K74-20R	817	142	46.49
K74-43H	381	49	38.64
$Total_3$	1198	191	X_t^2 =85.13
	X_d^2 =83.25		X_h^2 =1.88
$Total_3$	1198	191	85.13
K74-49H	825	72	128.43
	2023	263	X_t^2 =213.56
	X_d^2 =201.95		X_h^2 =11.61

Chi-square values are calculated using 25:8 as the expected ratio.
X_t^2 is the total of the Chi-square values for each class with 2df for $total_3$ and 3df for the overall total.
X_d^2 is the Chi-square value of the total of the classes with 1df.
X_h^2 is the difference between X_t^2 and X_d^2 and tests whether the classes fit the hypothesis and are segregating in the same ratio. Df_h for $total_3$=1. Df_h overall=2.

Table 11. Homogeneity test of the ratios of screening groups.

Group F_2 F_3	F_3 plant ratios Res.	Sus.	Chi-square
4 4	2817	401	243.90
3 4	1198	191	85.13
$Total_4$	4015	592	X_t^2 =329.03
	X_d^2 =325.73		X_h^2 =3.30
$Total_4$	4015	592	329.03
3 3	1132	262	25.38
	5147	854	354.41
	X_d^2 =327.70		X_h^2 =26.71

Chi-square values are calculated using 25:8 as the expected ratio.
X_t^2 is the total of the Chi-square values for each class with 5df for $total_4$ and 7df for the overall total.
X_d^2 is the Chi-square value of the total of the classes with 1df.
X_h^2 is the difference between X_t^2 and X_d^2 and tests whether the classes fit the hypothesis and are segregating in the same ratio. Df_h for $total_4$=4. Df_h overall=6.

The genotypes of PI54606-1 and FC31630 differ in regards to resistance to races 1,3 and 4. Determination of interrelationships between FC31630 and either PI 54606-1 or the Mukden source of resistance must await further study.

References

(1) Bernard, R. L., . E. Smith, M. J. Kaufmann and A. F. Schmitthenner. 1957. Inheritance of resistance to Phytophthora root and stem rot in the soybean. Agron. J. 49:391.

(2) Haas, J. H. and R. I. Buzzell. 1976. New races 5 and 6 of Phytophthora megasperma var. sojae and differential reactions of soybean cultivars to races 1 to 6. Phytopath. 66:1361-1362.

(3) Hartwig, E. E., B. L. Keeling and C. J. Edwards Jr. 1968. Inheritance of reaction to Phytophthora rot in the soybean. Crop Sci. 8:329-330.

(4) Kaufmann, M. J. and J. W. Gerdemann. 1958. Root and stem rot of soybeans by Phytophthora sojae N. Sp. Phytopath. 48:201-208.

(5) Kilen, T. C., E. E. Hartwig and B. L. Keeling. 1974. Inheritance of a second major gene for resistance to Phytophthora rot in soybeans. Crop Sci. 14:260-262.

(6) Lam-Sanchez, A., A. H. Probst, F. A. Laviolette, J. F. Schafer and K. L. Athow. 1968. Sources and inheritance of resistance to Phytophthora megasperma var. sojae in soybeans. Crop Sci. 8:329-330.

(7) Laviolette, F. A. and K. L. Athow. 1977. Three new physiologic races of Phytophthora megasperma var. sojae. Phytopath. 67:267-268.

(8) Morgan, F. L. and E. E. Hartwig. 1965. Physiologic specialization in Phytophthora megasperma var. sojae. Phytopath. 55:1277-1279.

(9) Schmitthenner, A. F. 1972. Evidence for a new race of Phytophthora megasperma var. sojae pathogenic to soybean. Plant Dis. Reptr. 56: 536-539.

(10) Schwenck, F. W. and T. Sim. 1974. Race 4 of Phytophthora megasperma var. sojae from soybeans proposed. Plant Dis. Reptr. 58:352-354.

(11) Suhovecky, A. J. and A. F. Schmitthenner. 1955. Soybeans affected by early root rot. Ohio Farm and Home Research 40:296.

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Abstract

The inheritance of host resistance to races 1, 3 and 4 of Phytophthora megasperma var. sojae in the plant introductions, PI54606-1 and FC31630, of soybeans was examined. PI54606-1 was crossed to the Mukden source of host resistance to determine if PI54606-1 contained a new source of host resistance to race 1. PI54606-1 and FC31630 were, also, crossed to lines susceptible to races 1, 3 and 4 to determine the genes for resistance. PI54606-1 was found to have a new locus for resistance to race 1. Resistance to races 3 and 4 were found to be controlled by a pair of linked loci, a dominant allele needed at each locus for resistance. The genotypes of FC31630 and PI54606-1 differ in regards to resistance to races 1, 3 and 4.