

Inheritance of male-sterility in soybean

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ABSTRACT

Genetic studies on a spontaneous male-sterile/female-fertile soybean mutant identified at the Embrapa Soybean breeding program were carried out in Londrina County, State of Paraná, South Brazil. The mutant showing segregation for male-sterility (BR97-17958) was selected within F₅ progeny lines derived from the BR5(6) x Paranaíba cross performed in 1995. The F₁, F₂ and F₃ generations from test-crossings among heterozygous plants of the BR97-17958 line and recessive homozygous plants (male-sterile) of the T 266H (*ms₁ms₁*), T 259H (*ms₂ms₂*), T 273H (*ms₃ms₃*), T 274H (*ms₄ms₄*), T 277H (*ms₅ms₅*) and T 295H (*ms₆ms₆*) lines were studied to identify whether this mutant defines a new locus or represents an independent mutation in one of the six loci already described. The F₁, F₂, and F₃ plants from the crosses were visually classified as presenting normal phenotype of male fertility or male-sterility. Results from inheritance and allele test study among the mutant genotypes and the recessive homozygous male-sterile lines (*ms₁*, *ms₂*, *ms₃*, *ms₄*, *ms₅* and *ms₆*) provided evidence that the male-sterile characteristic of the line BR97-17958 have a simple recessive Mendelian inheritance and represents a mutation in a locus different from the *ms*-loci already described.

KEY WORDS: *Glycine max*; male-sterile; genetics.

INTRODUCTION

Genetic male-sterility, although relatively rare, has been already observed in many species of autogamous (or self pollinated) plants. During the past three decades genetic and cytogenetic studies were carried out leading to the identification of six independent loci conditioning male-sterility in soybean [*Glycine max* (L.) Merr.], all of them controlled by recessive monogenic inheritance factors (Palmer et al., 1992). These studies have identified the alleles *ms₁ms₁* in the genetic type T 260H (Brim and Young, 1971), the alleles *ms₂ms₂* in T 259H (Bernard and Creemens, 1975), the alleles *ms₃ms₃* in T 273H (Palmer et al., 1980), the alleles *ms₄ms₄* in T 274H (Delannay and Palmer, 1982), the alleles *ms₅ms₅* in T 277H (Buss, 1983) and the alleles *ms₆ms₆* in T 295H (Skorupska and Palmer, 1989).

Several male-sterile genotypes were selected within segregating soybean populations at the Embrapa Soybean breeding program, in Londrina County, State of Paraná (PR), Brazil. Among these, the line BR97-17958 stands out due to its excellent agronomic performance. The knowledge of the genetic control of the male-sterility trait of this line may contribute to the use of this character in population breeding

programs and recurrent selection processes.

The objective of this study was to investigate the inheritance of the male-sterile trait of the genotype BR97-17958 and to carry out allele tests between this line and the known sources of genes conditioning male-sterility in order to verify whether this mutation defines a new locus or represents an independent mutation in the already described loci.

MATERIAL AND METHODS

The line BR97-17958, with male-sterile/female-fertile trait was found in a F₅ progeny selected in a segregating population of the BR5(6) x Paranaíba crossing, in 1995.

To investigate how the male-sterile trait of a spontaneous soybean mutant is inherited and to perform allele tests with gene loci already identified, the generations F₁, F₂ and F₃ resulting from the cross of the genotype BR97-17958 and the genetic types T 266H (Boerma and Cooper, 1978), T 259H (Bernard and Creemens, 1975), T 273H (Palmer et al., 1980), T 274H (Delannay and Palmer, 1982), T 277H (Buss, 1983) and T 295H (Skorupska and Palmer, 1989) were studied. T 266H was used due to its greater female fertility than T 260H. In obtaining generations

by self-pollination, the progeny of individual plants was maintained from F_1 to form F_2 and F_3 families, as in the genealogic method of homozygous advance. The experiment was carried out at Embrapa Soybean in Londrina, PR in the 1998, 1999 and 2000 growing seasons.

Allele tests were performed by crossing the mutant genotype with the known sources of genes in the ms -loci, in all possible combinations. BR97-17958 heterozygous plants were used as male parent in the crosses. As female parent, male-sterile plants of the American genetic types T 266H, T 259H, T 273H, T 274H, T 277H and T 295H were used (ms_1 , ms_2 , ms_3 , ms_4 , ms_5 and ms_6 , respectively). Seeds of the genetic types segregating for male-sterile trait and F_1 seeds that originated heterozygous plants of the BR97-17958 genotype were obtained at the Soybean Germplasm Bank maintained by Embrapa Soybean, in Londrina, PR.

The parents used in the crosses and the advanced generations for seed production of the F_2 population were cultivated in the greenhouse. The F_2 and F_3 generations were carried out in the field maintaining the individuality of each progeny. At maturity, the F_1 , F_2 , and F_3 plants from each progeny were visually classified as normal male-fertile phenotype or male-sterile. The frequency of plants in each class was recorded.

The chi-square test (χ^2) (LeClerg et al., 1939) was used to test the hypothesis of monogenic or digenic inheritance and to analyze the frequency distribution of plants in the two classes.

RESULTS AND DISCUSSION

Several different segregation rates may be expected in the F_1 and F_2 generations of the crosses studied. If the mutant gene is allelic to one of the already described genes for male-sterility, a 1:1 ratio of fertile and sterile plants is expected in the F_1 and a 3:1 ratio in the F_2 . When two heterozygous loci are involved in the independent segregation of two pairs of alleles, with complete dominance for fertility, no male-sterile plant is expected in the F_1 generation. In the F_2 generation a 1:1 ratio is expected of families presenting segregation ratios of 3:1 and 9:7.

Results obtained from the F_1 , F_2 and F_3 generations of the crosses involving the genotype BR97-17958 and the mutant male-steriles provided evidences that the mutation in the line is of a monogenic inheritance controlled by a pair of recessive alleles conditioning

male-sterility. These results are in agreement with the studies which identified six loci with recessive alleles conditioning male-sterility in soybean genotypes: ms_1 (Brim and Young, 1971); ms_2 (Bernard and Creemens, 1975); ms_3 (Palmer et al., 1980); ms_4 (Delannay and Palmer, 1982); ms_5 (Buss, 1983) and ms_6 (Skorupska and Palmer, 1989).

Male-sterile F_1 plants were not observed in the crosses involving T 266H, T 259H, T 273H, T 274H, T 277H and T 295H with the line (genotype) studied. The lack of male-sterile plants in the F_1 generation was an indication of the presence of one male sterility-inducer gene in a locus different from the ms_1 to ms_6 loci. In the segregation of each F_2 population, two classes of segregation were observed showing 3:1 and 9:7 segregation ratios.

In the T 266H x BR97-17958 cross, four F_2 families were evaluated from which one and three families showed segregation adjusted to the expected 9:7 and 3:1 segregation ratios, respectively, and with acceptable values of χ^2 and probability (Table 1). Results obtained in the segregation of the F_3 families of this testcross confirm that the mutation in BR97-17958 is controlled by one recessive gene, which is present in a locus different and independent of the ms_1 locus (Table 2). Results from inheritance and allele tests carried out by Seifert et al. (2001) between the BR93-12879 mutant genotype and the male-sterile (ms_1 , ms_2 , ms_3 , ms_4 , ms_5 and ms_6) provided evidences of simple recessive Mendelian inheritance, representing a gene mutation occurred in the ms -locus allelic to the ms_1 gene (T 266H).

The segregation of the T 259H x BR97-17958 cross was very similar to that obtained in the previously described cross. One and two F_2 families presented adjustment to the 9:7 and 3:1 expected segregation ratios, respectively (Table 1). These ratios of segregation indicated that the mutation in BR97-17958 occurred in a locus different and independent from the ms_2 -locus. This hypothesis was confirmed by the data obtained by the segregation of the F_3 families (Table 2). Genetic and cytological studies carried out by Bione et al. (2002) between the BR97-17971 mutant genotype and the male-sterile (ms_1 , ms_2 , ms_3 , ms_4 , ms_5 and ms_6), identified that the mutation in BR97-17971 was controlled by a recessive gene, representing a gene mutation occurred in the ms -locus allelic to the ms_2 gene (T 259H).

In the T 273H x BR97-17958 cross, four families presenting 129 fertile : 39 sterile segregation ratio and one family presenting 24 fertile : 15 sterile segregation ratio, that is 3:1 and 9:7, respectively,

were observed (Table 1). Results from the segregation of F_3 families of this test cross, provided evidences that the mutation in the BR97-17958 genotype occurred in a locus independent of the ms_3 -locus (Table 2).

The F_2 families from the allele test between T 274H x BR97-17958 segregated in two distinct classes. Six families segregated in a 3:1 ratio and four families a 9:7 segregation ratio (Table 1). The segregation was independent and non-allelic to the ms_4 -locus. This hypothesis was confirmed by the data obtained from the segregation of the F_3 families (Table 2).

Even though the analysis was carried out on three families of F_2 plants only, the same behavior was observed in the T 277H x BR97-17958 test cross that segregated two distinct classes. Two families segregated in a 3:1 ratio and one family in a 9:7 segregation ratio (Table 1). Based on these results, the male-sterile trait of the BR97-17958 genotype is conditioned by one non-allelic recessive gene, which is independent of the ms_5 -locus. This hypothesis was confirmed by data on segregation of F_3 families (Table 2).

In the F_2 generation of the T 295H x BR97-17958 test cross, two classes of segregation were observed, with progenies segregating individually at 3:1 and 9:7 ratios (Table 1). The high homogeneity and the significant adjustment in the expected 3:1 and 9:7 ratios were evidences that the mutation in the BR97-17958 genotype was non-allelic to the ms_6 -locus. Results were confirmed by data on segregation of F_3 families (Table 2).

The lack of F_1 male-sterile plants and the results from segregation tests of the F_2 families (Table 1) are indicatives of the independent segregation hypothesis in the mutant locus as related to the ms_1 , ms_2 , ms_3 , ms_4 , ms_5 and ms_6 loci. Results obtained in the segregation of F_3 families confirm this hypothesis (Table 2).

CONCLUSION

Results obtained from the genetic segregation studies carried out with the spontaneous mutant male-sterile/female-fertile BR97-17958 genotype showed that in this line male-sterility is of simple recessive

Table 1. Plant segregation of F_2 progenies crosses between the genotypes T 266H (ms_1), T 259H (ms_2), T 273H (ms_3), T 274H (ms_4), T 277H (ms_5) and T 295 H (ms_6) and the BR97-17958 line.

Cross combination	Number of plants			χ^2 (3:1) ²	P ³	Number of plants			χ^2 (9:7) ²	P ³
	Fertile	Sterile	df ¹			Fertile	Sterile	df ¹		
<i>ms₁ ms₁ x BR97-17958</i>										
Total			3	2.56	0.46					
Pooled	137	39	1	0.76	0.38	33	22	1	0.31	0.58
Homogeneity			2	1.80	0.41			-	-	-
<i>ms₂ ms₂ x BR97-17958</i>										
Total			2	0.78	0.68					
Pooled	139	40	1	0.67	0.41	40	26	1	0.25	0.62
Homogeneity			1	0.11	0.74			-	-	-
<i>ms₃ ms₃ x BR97-17958</i>										
Total			4	3.80	0.43					
Pooled	129	39	1	0.29	0.59	24	15	1	0.44	0.51
Homogeneity			3	3.51	0.32			-	-	-
<i>ms₄ ms₄ x BR97-17958</i>										
Total			6	2.63	0.85			4	3.30	0.51
Pooled	201	63	1	0.18	0.67	94	80	1	0.35	0.55
Homogeneity			5	2.45	0.78			3	2.95	0.40
<i>ms₅ ms₅ x BR97-17958</i>										
Total			2	0.42	0.81					
Pooled	191	59	1	0.26	0.61	60	44	1	0.09	0.76
Homogeneity			1	0.16	0.69			-	-	-
<i>ms₆ ms₆ x BR97-17958</i>										
Total			5	1.07	0.96			3	1.26	0.74
Pooled	173	52	1	0.43	0.51	85	56	1	0.93	0.33
Homogeneity			4	0.64	0.96			2	0.33	0.85

¹Degrees of Freedom; ²Chi-square test (χ^2); ³Probability.

Table 2. Plant segregation of F₃ progenies crosses between the genotypes T 266H (*ms*₁), T 259H (*ms*₂), T 273H (*ms*₃), T 274H (*ms*₄), T 277 H (*ms*₅) and T 295H (*ms*₆) and the BR97-17958 line.

Cross combination	Segregation 3:1					Segregation 9:7									
	Number of plants		df ¹	χ^2 (3:1) ²	P ³	Number of plants		df ¹	χ^2 (3:1) ²	P ³	Number of plants		df ¹	χ^2 (9:7) ²	P ³
	Fertile	Sterile				Fertile	Sterile				Fertile	Sterile			
<i>ms</i> ₁ <i>ms</i> ₁ x BR97-17958															
Total			-	-	-			-	-	-			-	-	-
Pooled	75	26	1	0.03	0.86	37	11	1	0.11	0.74	31	22	1	0.11	0.74
Homogeneity			-	-	-			-	-	-			-	-	-
<i>ms</i> ₂ <i>ms</i> ₂ x BR97-17958															
Total			-	-	-			-	-	-			-	-	-
Pooled	234	69	1	0.08	0.37	59	16	1	0.54	0.46	42	29	1	0.24	0.62
Homogeneity			-	-	-			-	-	-			-	-	-
<i>ms</i> ₃ <i>ms</i> ₃ x BR97-17958															
Total			3	0.70	0.87			-	-	-			-	-	-
Pooled	681	213	1	0.68	0.41	152	47	1	0.20	0.65	60	49	1	0.06	0.81
Homogeneity			2	0.02	0.99			-	-	-			-	-	-
<i>ms</i> ₄ <i>ms</i> ₄ x BR97-17958															
Total			2	0.25	0.88			2	0.11	0.95			2	0.99	0.61
Pooled	174	60	1	0.05	0.82	107	38	1	0.11	0.74	82	54	1	0.90	0.35
Homogeneity			1	0.20	0.65			1	0	1			1	0.09	0.76
<i>ms</i> ₅ <i>ms</i> ₅ x BR97-17958															
Total			2	0.29	0.86			-	-	-			-	-	-
Pooled	610	212	1	0.27	0.60	227	69	1	0.45	0.50	161	119	1	0.18	0.67
Homogeneity			1	0.02	0.89			-	-	-			-	-	-
<i>ms</i> ₆ <i>ms</i> ₆ x BR97-17958															
Total			2	0.62	0.73			2	0.27	0.87			2	1.22	0.54
Pooled	228	69	1	0.49	0.48	163	57	1	0.10	0.75	116	78	1	0.99	0.32
Homogeneity			1	0.13	0.72			1	0.17	0.68			1	0.23	0.63

¹Degrees of Freedom; ²Chi-square test (χ^2); ³Probability.

Mendelian inheritance originated from mutation occurring in a locus independent of the *ms*₁, *ms*₂, *ms*₃, *ms*₄, *ms*₅ and *ms*₆ loci.

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RESUMO

Herança da macho-esterilidade em soja

Estudos genéticos foram conduzidos em um mutante espontâneo de soja macho-estéril/fêmea-fértil identificado no programa de melhoramento da Embrapa Soja, em Londrina, PR. No teste de progênies de plantas-F₃ selecionadas na população do cruzamento BR5(6) x Paranaíba, em 1995, foi selecionada a linhagem BR97-17958 que apresentava segregação para plantas macho-estéreis. As gerações F₁, F₂ e F₃ de cruzamentos-teste entre plantas heterozigotas da linhagem BR97-17958 e plantas

homozigotas recessivas de tipos genéticos macho-estéreis foram estudadas com o objetivo de verificar se esse mutante define um novo loco ou se representa uma mutação independente em qualquer um dos seis locos já descritos. As fontes de genes macho-estéreis usadas como parentais femininos foram os tipos genéticos T 266H (*ms*₁*ms*₁), T 259H (*ms*₂*ms*₂), T 273H (*ms*₃*ms*₃), T 274H (*ms*₄*ms*₄), T 277H (*ms*₅*ms*₅) e T 295H (*ms*₆*ms*₆). As plantas F₁, F₂ e F₃ dos cruzamentos foram visualmente classificadas como apresentando fenótipo normal de fertilidade masculina ou macho-estéril. Os resultados obtidos no estudo de herança e alelismo entre a linhagem mutante e os tipos genéticos macho-estéreis em homozigose recessiva (*ms*₁, *ms*₂, *ms*₃, *ms*₄, *ms*₅ e *ms*₆) forneceram evidências de que a característica macho-estéril da linhagem BR97-17958 possui herança Mendeliana simples recessiva e representa uma mutação gênica não alélica aos locos-*ms* já descritos.

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