

## Genetic studies of a male-sterile, female-fertile soybean mutant

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### ABSTRACT

Studies on a new spontaneous male-sterile / female-fertile soybean mutant identified by the Embrapa Soybean breeding program were carried out in Londrina, PR. The mutant showing segregation for male-sterility (BR93-12879) was selected within  $F_4$  progeny lines derived from the IAS-5 (3) X OCEPAR 9-SS1 cross performed in 1993. The  $F_1$ ,  $F_2$  and  $F_3$  generations of cross among heterozygous plants of the BR93-12879 line and recessive homozygous plants (male-sterile) of the T 266H ( $ms_1ms_1$ ), T 259H ( $ms_2ms_2$ ), T 273H ( $ms_3ms_3$ ), T 274H ( $ms_4ms_4$ ), T 277H ( $ms_5ms_5$ ) and T 295H ( $ms_6ms_6$ ) lines were studied to identify whether the new mutation is conditioned by a new allele or by a mutation in one of the six loci already described in the literature. The  $F_1$ ,  $F_2$  and  $F_3$  plants from the crosses were visually classified as male-sterile or male-fertile. Results from the allele test and inheritance study among the mutant genotype and the recessive homozygous male-sterile lines ( $ms_1$ ,  $ms_2$ ,  $ms_3$ ,  $ms_4$ ,  $ms_5$  and  $ms_6$ ) showed that a single recessive gene controls the male-sterile trait of BR93-12879. This gene is allele to the already described  $ms_1$ -gene and resulted from a genetic mutation in the  $ms$ -loci.

**KEY WORDS:** *Glycine max*, genetics.

### INTRODUCTION

Male-sterile / female-fertile mutations are found in many cultivated plant species, but their detection in soybean (*Glycine max* (L.) Merrill) is relatively recent. Since the description of the first completely male-sterile mutant in soybean (Brim and Young, 1971), genetic and cytogenetic studies have identified six independent loci with pairs of recessive alleles conditioning male-sterility. The following alleles have been identified in these studies:  $ms_1ms_1$  in genotype T 260H (Brim and Young, 1971), the  $ms_2ms_2$  in T 259H (Bernard and Creemens, 1975), the  $ms_3ms_3$  in T 273H (Palmer et al., 1980),  $ms_4ms_4$  in T 274H (Delannay and Palmer, 1982),  $ms_5ms_5$  in T 277H (Buss, 1983) and  $ms_6ms_6$  in T 295H (Skorupska and Palmer, 1989).

The male-sterile / female-fertile trait may contribute to genetic studies and facilitate the production of many hybrid seeds necessary for breeding programs where recurrent selection is used.

Several male-sterile genotypes were selected within segregant soybean populations from the Embrapa Soybean breeding program at Londrina PR. BR93-12879 is a spontaneous mutation detected during the population development process and was selected due to its excellent agronomic performance.

This study was planned to investigate the inheritance of the BR93-12879 male-sterile trait. Allele tests between this line and the known sources of male sterility genes were carried out to check whether this mutation defines a new locus controlling the character or represents an independent mutation in the already described loci.

### MATERIALS AND METHODS

Allele tests were performed to investigate the inheritance of the male-sterile spontaneous mutation of BR93-12879. The male-sterile mutants  $ms_1$ ,  $ms_2$ ,  $ms_3$ ,  $ms_4$ ,  $ms_5$  and  $ms_6$  already identified, respectively, in T 266H (Boerma and Cooper, 1978), T 259H, T 273H, T 274H, T 277H e T 295H lines were used. T 266H was used instead of T 260H due to its greater female fertility. Line BR93-12879 was identified in 1993 in a  $F_4$  progeny test selected from the segregant population of the IAS-5(3) X OCEPAR 9-SS1 cross. BR93-12879 seeds segregated for the male-sterile trait and six lines were obtained from the Soybean Germplasm Bank of Embrapa Soybean at Londrina PR.

The  $F_1$ ,  $F_2$  and  $F_3$  generations derived from crosses involving BR93-12879 plants heterozygous for male sterility and the six lines were studied in

experiments carried out at Embrapa Soybean in 1998, 1999 and 2000. The heterozygous BR93-12879 plants were used as male parent in the crosses.

The parents used in the crosses and the  $F_1$  plants were cultivated in a greenhouse. The  $F_2$  and  $F_3$  generations were conducted in the field with progeny identification. At maturity, the  $F_1$ ,  $F_2$  and  $F_3$  plants of each progeny were visually classified as normal male-fertile phenotype or male-sterile. The frequencies of plants in each class was recorded.

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

## RESULTS AND DISCUSSION

Results obtained from the  $F_1$ ,  $F_2$  and  $F_3$  generations of crosses involving the BR93-12879 mutant and the known male-sterile lines showed that male sterility in the BR93-12879 is monogenic inherited controlled by a pair of recessive alleles that condition male-sterility.

These results are in agreement with studies that identified six loci with recessive alleles that condition male-sterility in soybean genotypes (Brim and Young, 1971; Bernard and Creemens, 1975; Palmer et al. 1980; Delannay and Palmer, 1982; Buss, 1983; Skorupska and Palmer, 1989).

Several segregation ratios may appear in the  $F_1$  and  $F_2$  generations of the studied crosses. If the mutant gene is allelic to one of the genes already described 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 in the  $F_2$ . If two independent heterozygous loci, each with two completely dominant alleles for fertility, are involved in the control of the trait, 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 3:1 and 9:7 segregation ratios of fertile and sterile plants.

The occurrence of male-sterile  $F_1$  plants was observed only in the T 266H x BR93-12879 cross at the ratio of seven normal to five sterile plants. The segregation in the  $F_1$  generation at the expected 1:1 ratio was an indication that the mutant is an allele in the  $ms_1$  locus of the T 266H line. In a total of 223 plants from the  $F_2$  population, 169

fertile and 54 male-sterile individuals were observed (Table 1). The high homogeneity and the non-significant deviation from the expected 3:1 segregation ratio proved that the male-sterile trait in BR93-12879 is inherited as a recessive gene segregating in the  $ms_1$  locus. These data were confirmed by the results from the  $F_3$  family segregation, where no significant difference was found between the expected and the observed 3:1 segregation ratio (Table 2). The available data did not allow any inference as to whether the mutation in BR93-12879 and the  $ms_1$  locus of T 266H carry identical alleles. Genetic studies by Brim and Young (1971); Palmer and Winger (1975); Boerma and Cooper (1978); Palmer et al. (1978); Yee and Jian (1983); Skorupska and Palmer (1987, 1988), reported seven independent mutations for the  $ms_1$  locus.

The  $F_2$  generation of the cross between the T 259H, T 273H, T 274H, T 277H and T 295H genotypes with the BR93-12879 tester line showed 3:1 and 9:7 segregation ratios among the progenies (Table 1). Results from the chi-square tests and the absence of male-sterile  $F_1$  plants were good indicators of  $ms_2$ ,  $ms_3$ ,  $ms_4$ ,  $ms_5$  and  $ms_6$  loci independent segregation. Segregation within the  $F_3$  families crosses confirms the hypothesis of no allelism among the referred loci (Table 2).

## CONCLUSION

The following conclusions can be drawn from the genetic segregation results obtained from the crosses involving the male-sterile / female-fertile BR93-12879 soybean line:

1. The male-sterile trait in the BR93-12879 line has single locus Mendelian inheritance controlled by recessive homozygote alleles.
2. The male-sterile mutant represents a mutation in the  $ms_1$  locus, allelic to the  $ms_1$  gene.

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**Table 1** – Plants segregation of F<sub>2</sub> progeny crosses between the genotypes T 266H (*ms*<sub>1</sub>), T 259H (*ms*<sub>2</sub>), T 273H (*ms*<sub>3</sub>), T 274H (*ms*<sub>4</sub>), T 277H (*ms*<sub>5</sub>), T 295H (*ms*<sub>6</sub>) and BR93-12879 (*ms*<sub>7</sub>) line.

| Cross combination  | Number of plants |         | df <sup>1</sup> | $\chi^2$ (3:1) <sup>2</sup> | P <sup>3</sup> | Number of plants |         | df | $\chi^2$ (9:7) | P    |
|--|------------------|---------|-----------------|-----------------------------|----------------|------------------|---------|----|----------------|------|
|  | Fertile          | Sterile |                 |                             |                | Fertile          | Sterile |    |                |      |
| <i>ms</i> <sub>1</sub> <i>ms</i> <sub>7</sub> x BR93-12879 |                  |         |                 |                             |                |                  |         |    |                |      |
| Total  |                  |         | 5               | 3,47                        | 0,63           |                  |         |    |                |      |
| Pooled   | 169              | 54      | 1               | 0,07                        | 0,79           |                  |         |    |                |      |
| Homogeneity  |                  |         | 4               | 3,40                        | 0,49           |                  |         |    |                |      |
| <i>ms</i> <sub>2</sub> <i>ms</i> <sub>2</sub> x BR93-12879 |                  |         |                 |                             |                |                  |         |    |                |      |
| Total  |                  |         | 4               | 1,61                        | 0,81           |                  |         | 3  | 1,62           | 0,65 |
| Pooled   | 142              | 44      | 1               | 0,18                        | 0,67           | 109              | 74      | 1  | 0,81           | 0,37 |
| Homogeneity  |                  |         | 3               | 1,43                        | 0,70           |                  |         | 2  | 0,81           | 0,67 |
| <i>ms</i> <sub>3</sub> <i>ms</i> <sub>3</sub> x BR93-12879 |                  |         |                 |                             |                |                  |         |    |                |      |
| Total  |                  |         | -               | -                           | -              |                  |         | 3  | 2,18           | 0,54 |
| Pooled   | 45               | 12      | 1               | 0,47                        | 0,49           | 202              | 145     | 1  | 0,54           | 0,46 |
| Homogeneity  |                  |         | -               | -                           | -              |                  |         | 2  | 1,64           | 0,44 |
| <i>ms</i> <sub>4</sub> <i>ms</i> <sub>4</sub> x BR93-12879 |                  |         |                 |                             |                |                  |         |    |                |      |
| Total  |                  |         | 2               | 0,85                        | 0,65           |                  |         | 3  | 1,90           | 0,59 |
| Pooled   | 89               | 24      | 1               | 0,85                        | 0,36           | 128              | 55      | 1  | 0,01           | 0,92 |
| Homogeneity  |                  |         | 1               | 0                           | 1              |                  |         | 2  | 1,89           | 0,39 |
| <i>ms</i> <sub>5</sub> <i>ms</i> <sub>5</sub> x BR93-12879 |                  |         |                 |                             |                |                  |         |    |                |      |
| Total  |                  |         | 3               | 0,23                        | 0,97           |                  |         | 2  | 0,95           | 0,62 |
| Pooled   | 126              | 42      | 1               | 0                           | 1              | 83               | 61      | 1  | 0,11           | 0,74 |
| Homogeneity  |                  |         | 2               | 0,23                        | 0,89           |                  |         | 1  | 0,84           | 0,36 |
| <i>ms</i> <sub>6</sub> <i>ms</i> <sub>6</sub> x BR93-12879 |                  |         |                 |                             |                |                  |         |    |                |      |
| Total  |                  |         | 2               | 0,66                        | 0,72           |                  |         | 4  | 2,76           | 0,60 |
| Pooled   | 77               | 25      | 1               | 0,01                        | 0,92           | 153              | 102     | 1  | 1,46           | 0,23 |
| Homogeneity  |                  |         | 1               | 0,65                        | 0,42           |                  |         | 3  | 1,30           | 0,73 |

<sup>1/</sup> Degrees of Freedom; <sup>2/</sup> Chi-square test ( $\chi^2$ ); <sup>3/</sup> Probability.

**Table 2.** Plants segregation of F<sub>3</sub> progeny crosses between the genotypes T 266H (*ms*<sub>1</sub>), T 259H (*ms*<sub>2</sub>), T 273H (*ms*<sub>3</sub>), T 274H (*ms*<sub>4</sub>), T 277H (*ms*<sub>5</sub>), T 295H (*ms*<sub>6</sub>) and BR93-12879 (*ms*<sub>7</sub>) line.

| Cross combination  | Segregation 3:1  |         |                 |                             |                | Segregation 9:7  |         |      |                |      |                  |      |      |                |      |
|--|------------------|---------|-----------------|-----------------------------|----------------|------------------|---------|------|----------------|------|------------------|------|------|----------------|------|
|  | Number of plants |         | df <sup>1</sup> | $\chi^2$ (3:1) <sup>2</sup> | P <sup>3</sup> | Number of plants |         | df   | $\chi^2$ (3:1) | P    | Number of plants |      | df   | $\chi^2$ (9:7) | P    |
| Fertile  | Sterile          | Fertile |                 |                             |                | Sterile          | Fertile |      |                |      | Sterile          |      |      |                |      |
| <i>ms</i> <sub>1</sub> <i>ms</i> <sub>7</sub> x BR93-12879 |                  |         |                 |                             |                |                  |         |      |                |      |                  |      |      |                |      |
| Total  |                  |         | 5               | 1,29                        | 0,94           |                  |         |      |                |      |                  |      |      |                |      |
| Pooled   | 849              | 266     | 1               | 0,78                        | 0,38           |                  |         |      |                |      |                  |      |      |                |      |
| Homogeneity  |                  |         | 4               | 0,51                        | 0,97           |                  |         |      |                |      |                  |      |      |                |      |
| <i>ms</i> <sub>2</sub> <i>ms</i> <sub>2</sub> x BR93-12879 |                  |         |                 |                             |                |                  |         |      |                |      |                  |      |      |                |      |
| Total  |                  |         | -               | -                           | -              | 3                | 1,48    | 0,69 |                |      | 3                | 1,24 | 0,74 |                |      |
| Pooled   | 95               | 30      | 1               | 0,07                        | 0,79           | 313              | 91      | 1    | 1,32           | 0,25 | 222              | 157  | 1    | 0,83           | 0,36 |
| Homogeneity  |                  |         | -               | -                           | -              | 2                | 0,16    | 0,92 |                |      | 2                | 0,41 | 0,81 |                |      |
| <i>ms</i> <sub>3</sub> <i>ms</i> <sub>3</sub> x BR93-12879 |                  |         |                 |                             |                |                  |         |      |                |      |                  |      |      |                |      |
| Total  |                  |         | -               | -                           | -              | 3                | 0,78    | 0,85 |                |      | 3                | 0,23 | 0,97 |                |      |
| Pooled   | 131              | 40      | 1               | 0,24                        | 0,62           | 497              | 162     | 1    | 0,06           | 0,81 | 495              | 394  | 1    | 0,12           | 0,73 |
| Homogeneity  |                  |         | -               | -                           | -              | 2                | 0,72    | 0,70 |                |      | 2                | 0,11 | 0,95 |                |      |
| <i>ms</i> <sub>4</sub> <i>ms</i> <sub>4</sub> x BR93-12879 |                  |         |                 |                             |                |                  |         |      |                |      |                  |      |      |                |      |
| Total  |                  |         | -               | -                           | -              | 2                | 0,05    | 0,98 |                |      | 2                | 0,44 | 0,80 |                |      |
| Pooled   | 121              | 43      | 1               | 0,13                        | 0,72           | 290              | 99      | 1    | 0,04           | 0,84 | 219              | 177  | 1    | 0,14           | 0,71 |
| Homogeneity  |                  |         | -               | -                           | -              | 1                | 0,01    | 0,92 |                |      | 1                | 0,30 | 0,58 |                |      |
| <i>ms</i> <sub>5</sub> <i>ms</i> <sub>5</sub> x BR93-12879 |                  |         |                 |                             |                |                  |         |      |                |      |                  |      |      |                |      |
| Total  |                  |         | 2               | 0,25                        | 0,88           |                  |         | 2    | 0,22           | 0,90 |                  |      | 2    | 0,15           | 0,93 |
| Pooled   | 374              | 122     | 1               | 0,04                        | 0,84           | 285              | 94      | 1    | 0,01           | 0,92 | 241              | 186  | 1    | 0,01           | 0,92 |
| Homogeneity  |                  |         | 1               | 0,21                        | 0,65           |                  |         | 1    | 0,21           | 0,65 |                  |      | 1    | 0,14           | 0,71 |
| <i>ms</i> <sub>6</sub> <i>ms</i> <sub>6</sub> x BR93-12879 |                  |         |                 |                             |                |                  |         |      |                |      |                  |      |      |                |      |
| Total  |                  |         | 2               | 0,11                        | 0,95           |                  |         | 3    | 0,30           | 0,96 |                  |      | 3    | 0,69           | 0,88 |
| Pooled   | 235              | 76      | 1               | 0,05                        | 0,82           | 192              | 63      | 1    | 0,05           | 0,82 | 137              | 96   | 1    | 0,62           | 0,43 |
| Homogeneity  |                  |         | 1               | 0,06                        | 0,81           |                  |         | 2    | 0,25           | 0,88 |                  |      | 2    | 0,07           | 0,97 |

<sup>1/</sup> Degrees of Freedom; <sup>2/</sup> Chi-square test ( $\chi^2$ ); <sup>3/</sup> Probability.

## RESUMO

### Estudos genéticos de um mutante macho-estéril, fêmea-fértil em soja

Foram conduzidos estudos genéticos com um novo mutante espontâneo de soja macho-estéril / fêmea-fértil identificado no programa de melhoramento da Embrapa Soja, em Londrina-PR. O mutante apresentando segregação para macho-esterilidade (BR93-12879), foi selecionado em linhagens de teste de progênies, plantas-F<sub>4</sub>, provenientes do cruzamento IAS-5(3) X OCEPAR 9-SS1 realizado

em 1993. As gerações F<sub>1</sub>, F<sub>2</sub> e F<sub>3</sub> de cruzamentos entre plantas heterozigotas da linhagem BR93-12879 e plantas homozigotas recessivas (macho-estéreis) das linhagens T 266H (*ms*<sub>1</sub>*ms*<sub>1</sub>), T 259H (*ms*<sub>2</sub>*ms*<sub>2</sub>), T 273H (*ms*<sub>3</sub>*ms*<sub>3</sub>), T 274H (*ms*<sub>4</sub>*ms*<sub>4</sub>), T 277H (*ms*<sub>5</sub>*ms*<sub>5</sub>) e T 295H (*ms*<sub>6</sub>*ms*<sub>6</sub>) foram estudadas objetivando identificar se a nova mutação é condicionada por um novo alelo ou por uma mutação ocorrida em um dos seis locos já descritos. As plantas F<sub>1</sub>, F<sub>2</sub> e F<sub>3</sub> dos cruzamentos foram visualmente classificadas como apresentando fenótipo normal de fertilidade masculina ou macho-estéril. Os resultados obtidos no teste de alelismo e estudo de herança

entre o genótipo mutante e as linhagens 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 BR93-12879 possui herança mendeliana simples recessiva e representa uma mutação gênica ocorrida no locus  $ms$ , alélica ao gene  $ms_1$  já descrito.

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