Genetic Analysis of Soybean Seed Response to Mechanical Damage

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ABSTRACT

Soybean (*Glycine max (L.) Merrill*) seed susceptibility to mechanical damage during harvesting, threshing, and storage operations has decreased seed quality by reducing seed germination ability and seedling vigour, mainly in tropical and subtropical environments. This research aimed at estimating the genetic variability of seed response to mechanical damage (MD), and its relationship to agronomic characters such as plant height at maturity (PHM) and seed yield (SY). The material included 10 early maturity parents and 45 diallel crosses among them and five experimental checks. Ten $F_{8:5}$ experimental lines from each cross were evaluated. The statistical and genetical analyses included estimates of phenotypic and genotypic correlations; relative contribution of genetic effects versus combined genetic and environmental effects; relationship between the genetical and experimental variation coefficients; coefficient of genotypic determination; expected and observed gain; and, correlated response to selection. The parents Paraná, Hale-321, IAC-Foscarin-31 and Primavera, showed the same MD resistance levels as IAS-5 cultivar, a standard check for MD resistance. The parents Palmetto, Coker-136, FT-81-1835 and Forrest were MD susceptible; however, they were superior to the IAC-2 cultivar, a standard check for MD susceptibility. The coefficient of genotypic determination for MD was estimated in 0.739, indicating large possibility to select MD resistant inbred lines in a population represented by the 45 diallel crosses. Expected and observed genetic gains indicated the possibility of success in the selection for resistance to MD among and within crosses. Based on the low genetic correlations (<0.25) among MD, PHM, and SY, all possible combinations of these traits could be found in a same soybean plant, at least in the early maturity group.

KEY WORDS: *Glycine max*, Genetic variability, Genetic gains, Breeding.

INTRODUCTION

Soybean seeds are very sensitive to mechanical damage (MD). The vital parts of the embryonary axis (radicle, hypocotyl and plumule) are located under a very thin seed coat, which offers little protection (Copeland, 1972; Delouche, 1972; Gupta *et al.*, 1973; França Neto and Henning, 1984; Costa *et al.*, 1986). MD causes reduction of seed vigor and germination. Damage also promotes hydrolysis and oxidation of fatty acids, which give the grains an unpleasant odor and flavor (Rodda *et al.*, 1972).

MD resistance varies genetically in the soybean germplasm. Evaluation of MD methods appropriate for screening genotypes for MD resistance is a recent occurrence. Few papers deal with selection methods such as the drop test (Dickson and Boettger, 1977; Kueneman, 1989 and Carbonell *et al.*, 1992), stationary threshers (Costa *et al.*, 1987), the pendulum test (Carbonell, 1991) and the correlation between lignin content in the seed coat and its reaction to MD (Alvarez, 1994). Dickson and Boettger (1977) have studied the genetical aspects of MD using *Phaseolus vulgaris* beans. They obtained heritability estimates from 22 to 73%. No report of MD heritability in soybeans was found in the literature.

The pendulum test methodology originally proposed by Carbonell (1991) is composed of specific field procedures and laboratory handling, which are summarized as follows: (1) manual harvesting of plants at physiological maturity stage (R_7); (2) manual threshing of plants; (3) drying of seeds until 13% $\pm$ 0.5% of moisture; (4) manual cleaning and uniformizing of the seed size through

a set of sieves; (5) removing of the seeds damaged by stink bugs, machines or weather; (6) sampling of 100 seeds; (7) dropping a metal pendulum from the height of 13 cm onto individual seeds placed on a metal plate to cause mechanical damage; (8) evaluating the mechanical damage by tetrazolium and/or chlorox tests, and (9) quantifying MD through the analysis of the magnitude of seed damage.

The objectives of this research was to estimate the genetic variability of the MD character, its relationships with plant height at maturity, and seed yield.

MATERIAL AND METHODS

Ten inbred $F_{8:5}$ lines from each of the 45 crosses were evaluated. These lines were developed by the Department of Genetics, ESALQ, USP (Vello, 1992) using diallel crosses of the following 10 early maturity soybean parents: Paraná, IAC-Foscarin 31, Hale-321, Palmetto, Coker-136, SOC-81-79, Década, Forrest, Primavera, and FT-81-1835. Cultivars Paraná, IAC-Foscarin-31, Primavera and IAS-5 were used as checks for both cycle and seed yield in the field experiment. Cultivars IAS-5 and IAC-2 were used respectively as checks for MD resistance and susceptibility (Carbonell, 1991), in the laboratory evaluations.

The field experiment was designed in complete randomized block with four replications of 464 treatments: 10 $F_{8:5}$ experimental lines from each one of the 45 crosses, 10 parents, and four checks. The experimental plot consisted of a 2.0 x 0.50 m row. The experiment was sown on December 2, in Piracicaba, State of São Paulo.

The following characters were evaluated on a plot base:

PHM: plant height at maturity, which is measured in centimeters from the base of the plant to the top of the main stem;
SY: seed yield, which is the weight in grams of the grains in the plot (g/m^2);

MD: mechanical damage, which is the damage caused on the seed by dropping a pendulum on the seed; estimated by the weighted mean values of a sample with 50 seeds.

Preparation of seed samples

Five plants were harvested just at the morphological maturity stage (R_8). The plants were threshed immediately in a wooden box by gently beating the plants against the internal walls. These two modifications of harvesting at R_8 and altering the threshing method improved the methodology originally proposed by Carbonell (1991). As a result, seed processing was made easier and quicker.

Seed samples from each inbred line were conditioned in paper bags and placed in a drying chamber at 26°C and 40% of relative air moisture-RH for approximately four months, resulting in moisture uniformity of 7% (equilibrium moisture). After the initial conditioning, the seeds were removed from the drying chamber and left overnight at room temperature and moisture levels. This allowed the seeds to absorb the initial moisture slowly and avoid seed damage due to rapid swelling.

Then, the seeds were homogenized by size before taking a sample of 50 seeds from each treatment. Uniformity is important, because seed mass can be variable and cause differences in absorption of the falling pendulum's kinetic energy (Burrris, 1979). Homogeneity was obtained by passing the seeds through a round hole sieve (5.5 mm diameters hole) with a retention limit of 5 mm. These seeds were then subjectively analyzed visually to remove all seeds damaged by stink bug, machines or weather, which may interfere in the MD procedure's results. The selected samples (50 seeds from each treatment) were placed in a growing chamber at 25°C and 85% RH. After 72 h, a moisture content of approximately 13% was reached. According to Carbonell *et al.* (1993), the best seed moisture content for screening and classifying genotypes for MD resistance is 13% ± 0.5.

Mechanical damage

The pendulum test involves the use of an equipment (Figure 1) which drops a metal pendulum onto individual seeds placed on an indented metal plate.

Just one drop of the pendulum is enough to cause damage. However, the precise impact point on the seed is random and is considered an uncontrollable factor.
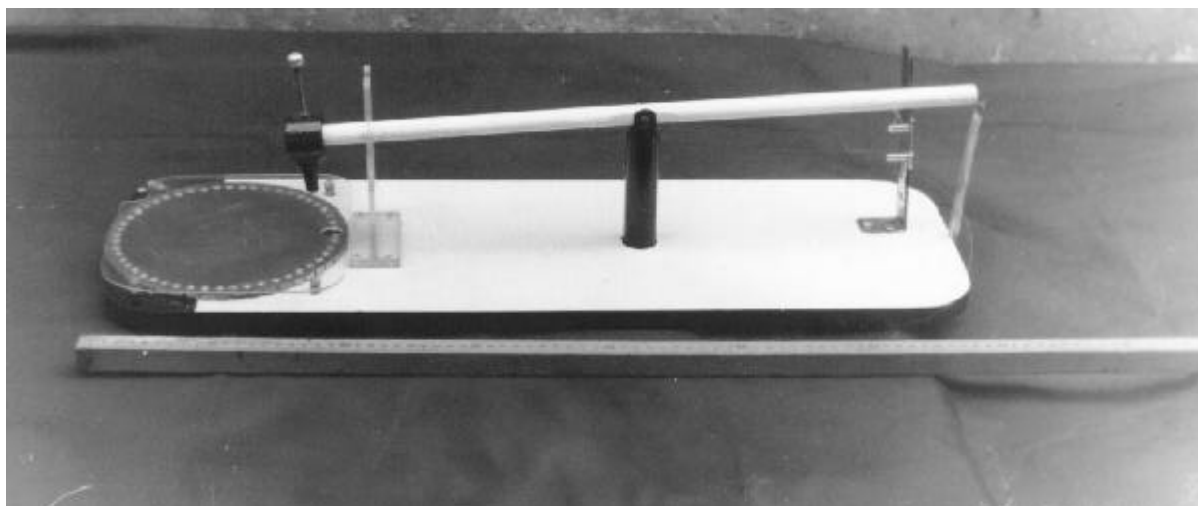
Figure 1 - Equipment used to cause mechanical damage on soybean seeds in the 'pendulum test'.ESALQ/ USP. Piracicaba, SP.

The height (13 cm) of the drop was previously determined by Carbonell and Krzyzanowski (1995). They determined that this height best separated genotypes into resistance classes. However, due to modification of the original method in recovering seeds from the field, pendulum height was reduced to 11 cm, which the authors agree is also efficient in screening genotypes.

The MD caused by the pendulum was evaluated by the chlorox test, since it is an easy, cost efficient, and quick test (Carbonell *et al.*, 1993). This test identifies cracks in the seed coat, through observing cotyledon swelling triggered by quick penetration of the solution (5 to 10 min) (Vaughan, 1982). Soaking time was extended to 10 to 15 min, and a 5.25% chlorox stock was diluted in distilled water in proportion of 25 ml to 975 ml to get the working solution. According to Carbonell *et al.* (1993), a longer soaking period better detects

small ruptures on the seed coats.

Procedure to quantify mechanical damage

After causing damage, the seeds were visually analyzed, and all broken and cracked seeds were separated. The chlorox test was used to detect small cracks in the seed coats of the apparently undamaged seeds. In order to obtain an average MD value, a susceptibility index was created for all seeds after the damage, as follows:

Seed class	Susceptibility index
Broken seeds (BS)	10
Damaged seeds (DS)	6
Swollen seeds (SS)	2
Non-swollen seeds	0
(no damage) (NSS)	

where:

- Broken seeds (BS): are broken or smashed seeds;
- Damaged seeds (DS): are seeds with visible damage, but do not have broken or partially broken cotyledons;
- Swollen seeds (SS): are seeds with little damage, invisible to the naked eye, but visible with the chlorox test;
- Non-swollen seeds (NSS): undamaged seeds that do not swell in the chlorox test.

By using these classes and indeces, an unique weighted value can be determined, which is expressed in units of MD (mdu). The MD values vary between zero and ten, with zero corresponding to maximum resistance and ten to maximum susceptibility:

$$MD = \frac{BS \times 10 + DS \times 6 + SS \times 2 + NSS \times 0}{BS + DS + SS + NSS}$$

where MD is the value of mechanical damage (mdu) and BS, DS, SS and NSS are the number of broken, damaged, swollen and non-swollen seeds, respectively.

Statistical and genetical analyses

The experimental plot data was analyzed using the statistical computer package SAS (SAS, 1982). A mixed mathematical model was used in these analyses. Parent and control observations had fixed effects and replications and experimental lines had random effects. The sample of 10 experimental lines was considered as representative of the maximum potential of each cross.

Relative contributions of genetic effects versus combined genetic and environmental effects were calculated for all 450 experimental lines, by using the estimates of coefficient of genotypic determination, as indicated by Falconer (1989), for a fixed parameter.

The relationship among the coefficients of genotypic

and experimental variation (b), the expected selection progress ($\hat{G}_S$) and the correlated response to selection (Rs) among the experimental lines, were obtained according to the formula reported by Vencovsky and Barriga (1992).

Observed genetic progress (G_s) was calculated for MD in each one of the 45 crosses by using the following equations:

$$\overline{G}_{sp} = \frac{\overline{x}_{cross} - \overline{x}_{parents}}{\overline{x}_{parents}} \cdot 100$$

$$\overline{G}_{sp_s} = \frac{\overline{x}_{cross} - \overline{x}_{ps}}{\overline{x}_{ps}} \cdot 100$$

where:

$\overline{G}_{sp}$: observed genetic progress based on the mean of the parents involved in the cross;

$\overline{G}_{sp_s}$: observed genetic progress based on the mean of the superior parent, that is, the parent with the smaller value of DM;

$\overline{X}_{cross}$: mean of the 10 experimental lines of each cross;

$\overline{X}_{parents}$: mean of the two parents of each cross;

$\overline{X}_{ps}$: mean of the superior parent.

Phenotypic (r_F), genotypic (r_G) and environmental (r_E) correlation coefficients among MD, PHM, and SY were calculated only for the experimental lines, through the respective variance and covariance estimates (Kempthorne, 1969).

RESULTS AND DISCUSSION

The MD mean for experimental lines was 3.8 mdu with a coefficient of variation (CV) of 24.6% (Table 1). This CV is considered acceptable. According to Paulsen and Nave (1977), values greater than 15% are common for studies of this nature.

The cultivars Paraná, Hale-321 and IAC-Foscarin-31 showed the smallest MD means, and consequently, higher MD resistance (Table 1). However, the cultivars Palmetto, Coker-136, FT-81-1835 and Forrest presented the highest MD values and the highest susceptibility to MD. In the check group, the cultivar IAS-5 had an MD resistance level similar (MD = 3.24 mdu) to that of the best parent, which was considered by Carbonell (1991) as a standard for damage resistance. Furthermore, the cultivar IAC-2, considered by Carbonell (1991) as standard for susceptibility, presented the highest values (MD = 5.52 mdu).

Based on the mean values, the three groups of genotypes were similar in DM: checks (3.55 mdu), experimental lines (3.8 mdu), and parents (3.89 mdu). The highest level of resistance to MD occurred in the crosses:

> Palmetto x Primavera (2.53 mdu)
> FT 81-1835 x Palmetto (2.62 mdu)
> Coker-136 x FT 81-1835 (2.87 mdu)
> Primavera x FT 81-1835 (2.95 mdu)

By considering all 450 experimental lines, relationship between genetic and experimental variation was estimates in b = 0.84 for MD (Table 1). In general terms, this estimate indicate medium probability of success in the selection for resistance to MD, once the ideal situation would occur with b ≥ 1 (Vencovsky and Barriga, 1992). Thus, the most promising crosses were:

> Forrest x Primavera (b = 1.38);
> Forrest x Década (b = 1.34);
> FT 81-1835 x Hale-321 (b = 1.17);
> Coker-136 x FT 81-1835 (b = 1.05).

Another indicator of success in the selection for resistance to MD is the magnitude of the coefficient of genotypic determination (H = 0.74) estimated for the population represented by 450 experimental lines (Table 1).

By using a selection intensity of 10% of genotypes

with smaller MD values, and consequently, higher damage resistance, the expected selection progress ($\hat{G}_s$) was 0.95 mdu (Table 1) among all 450 experimental lines. This value was considered high for MD, since it represents a gain of 24.4% on average. The highest estimates $\hat{G}_s$ occurred in the crosses Forrest x Primavera (1.99 mdu) and Forrest x Década (1.82 mdu).

The real potentiality of each cross can be provided by the observed genetic progress. By selecting the best experimental line (lowest MD values or highest resistance to MD) of each cross, the same selection intensity of 10% used before for $\hat{G}_s$ was obtained. Based on the observed genetic progress, about 50% (22/45) and 25% (11/45) of the crosses showed potentiality of success in the selection for resistance to MD, as indicated in Table 1 by the negative values of $\overline{G}_{sp}$ and $\overline{G}_{sp_s}$, respectively. The most outstanding crosses for both $\overline{G}_{sp}$ and $\overline{G}_{sp_s}$ were:

> Coker-136 x FT-81-1835
> Palmetto x Primavera
> Coker-136 x Palmetto
> Coker-136 x Soc 81-79
> Forrest x Palmetto, and
> FT 81-1835 x Palmetto

The correlation coefficients among characters were of low magnitude (<0.25, Table 2), in the population represented by all 450 experimental lines. In analysing Table 2, two additional points can be emphasized: firstly, the major importance of the genetic component (>70%) related to the environmental component (<30%) of the phenotypic correlations; secondly, the negative signal of the correlations involving the MD character. Thus, in selecting for resistance to MD (lowest correlations values) there would be none or just some small alterations on the PHM and SY. Small alterations would occur on PHM and SY in the desirable direction to increasing these characters, a fact that would be a major advantage mostly in breeding programs involving selection for early maturity soybeans.

Table 1 – MD: mechanical damage. Mean (mdu), number of observations included in the mean (N), coefficient of genotypic determination (H), relationship between genetical and environmental variation coefficients (b), expected selection progress ($\hat{G}_s$) and observed genetic progress based on the parental mean ($\overline{G}_{sp}$ %) and on the superior parent ($\overline{G}_{sp_s}$ %). Early maturity soybeans.

Group	Mean (mdu)	Nobs	b	$\hat{G}_s$	$\overline{G}_{sp}$	$\overline{G}_{sp_s}$
Parents:	3.89	40				
1. Paraná	1.76	4				
2. IAC -Foscarin-31	3.01	4				
3. Coker-136	4.91	4				
4. Primavera	3.20	4				
5. Década	3.90	4				
6. Forrest	4.65	4				
7. FT 81-1835	4.78	4				
8. SOC 81-79	4.51	4				
9. Hale-321	2.91	4				
10. Palmetto	5.26	4				
Checks:	3.55	16				
IAS-5	3.24	4				
Paraná	3.22	4				
Primavera	3.21	4				
IAC-Foscarin-31	4.51	4				
IAC-2	5.52	4				
Crosses:	3.80	1800	0.84	0.95		
1x2	4.67	40	0.32	0.31	95.89	165.45
1x3	3.75	40	0.00	0.00	12.35	112.90
1x4	3.27	40	0.62	0.51	31.77	85.68
1x5	4.01	40	0.66	0.74	49.07	127.84
1x6	4.15	40	0.82	1.03	29.48	135.79
1x7	3.23	40	0.33	0.18	-1.04	83.86
1x8	3.87	40	0.54	0.53	23.44	119.89
1x9	4.37	40	0.00	0.00	87.20	148.35
1x10	4.50	40	0.87	0.89	-0.54	98.35
2x3	3.45	40	0.64	0.58	-12.83	14.68
2x4	4.04	40	0.49	0.50	30.24	34.35
2x5	3.57	40	0.00	0.00	7.60	18.50
2x6	4.14	40	0.15	0.07	8.01	37.44
2x7	3.69	40	0.49	0.43	-5.34	22.49
2x8	3.78	40	0.66	0.69	0.53	25.58
2x9	5.10	40	0.60	0.89	72.20	75.15
2x10	3.51	40	0.56	0.49	-15.09	16.64
3x4	3.84	40	0.58	0.59	-5.30	20.00
3x5	3.86	40	0.73	0.81	-9.52	6.60
3x6	4.05	40	0.11	0.03	-15.31	-12.95
3x7	2.87	40	1.05	0.88	-40.66	-39.85
3x8	3.07	40	0.75	0.62	-34.86	-31.97
3x9	3.43	40	0.38	0.25	-12.15	18.04
3x10	3.28	40	0.38	0.24	-35.54	-33.24
4x5	4.05	40	0.42	0.40	18.80	26.59
4x6	4.39	40	1.38	2.00	11.77	37.09
4x7	2.95	40	0.70	0.53	-25.94	-7.66
4x8	3.87	40	0.53	0.52	0.47	21.03
4x9	3.82	40	0.77	0.86	24.94	31.17
4x10	2.53	40	0.50	0.24	-40.26	-21.03
5x6	4.16	40	1.34	1.82	0.70	15.03
5x7	3.53	40	0.61	0.57	-15.95	-2.49
5x8	3.76	40	0.46	0.41	-7.43	3.95
5x9	4.22	40	0.50	0.53	29.25	45.02
5x10	3.90	40	0.71	0.79	-12.07	7.84
6x7	5.18	40	0.21	0.19	9.88	11.42
6x8	4.61	40	0.87	1.25	0.63	2.19
6x9	4.35	40	0.42	0.43	15.18	49.62
6x10	3.23	40	0.42	0.28	-34.77	-30.49
7x8	3.67	40	0.30	0.20	-20.86	-18.49
7x9	4.09	40	1.17	1.53	6.29	40.45
7x10	2.62	40	0.66	0.40	-31.86	-9.97
8x9	3.51	40	0.00	0.00	-5.36	20.65
8x10	4.01	40	0.72	0.83	-17.93	-11.11
9x10	3.95	40	0.64	0.69	-3.23	35.84
CVexperimental lines (%) = 24.60			H = 0.74			

In spite of the low correlation values among MD, PHM and SY (Table 2), the correlated response was calculated (i = 10%) among and within crosses (Table 3).

The correlated response to selection (Rs) was considered high for all trait combinations. Values varied from 3.19% for SY:PHM to -7.15% for MD:SY. In fact, such values are relatively high if one considers the soybean's narrow genetic base (Hiromoto and Vello, 1986) and the relatively small group of genotypes with early maturity.

While selecting for higher MD resistance (low values MD), some crosses presented higher Rs for PHM and SY. The Rs values varied between -12.15% and 14.21% for PHM, and between -37.26% and 26.25% for SY. While selecting for higher plant height (PHM), some crosses showed higher Rs for MD resistance and SY: between -30.57% and 21.83%

Table 2 - Estimates of phenotypic (r_p), genotypic (r_g) and environmental (r_e) correlation; relative contribution of genetic effect versus combined genetic and environmental effects in phenotypic correlation of the pairs of characters: mechanical damage (MD), plant height at maturity (PHM) and seed yield (SY). Total of 45 crosses. Early maturity soybeans.

Group[1]	MD x PHM			MD x SY			PHM x SY		
	r_p	r_g	r_e	r_p	r_g	r_e	r_p	r_g	r_e
Experimental lines [2]	-0.18012	-0.24064	-0.00579	-0.17102	-0.22777	-0.01592	0.18592	0.16810	0.2499'
Relative contribuition (%)		99.18	0.82		97.54	2.46		70.71	29.29

[1] Estimates obtained with three replications.

[2] Number of observations = 1350

Table 3 - Correlated response to selection (Rs) and percentage (%) obtained in Rs involving mechanical damage (MD), plant height at maturity (PHM) and seed yield (SY), using 10% of selection intensity among experimental lines, and Rs variation (%) of the 45 crosses. Early maturity soybean.

Group[1]	N	Mean			MD:PHM[2]		PHM:MD		MD:SY		SY:MD		PHM:SY		SY:PHM	
		MD	PHM	SY	Rs	%	Rs	%	Rs	%	Rs	%	Rs	%	Rs	%
		umd	cm	g/m^2	cm		umd		g/m^2		umd		g/m^2		cm	
Experim. Lines	1350	3.49	73.71	137.37	-3.18	-4.34	-0.25	-7.15	-9.86	-7.18	-0.23	-6.68	7.77	5.65	2.34	3.19
Variation of Rs (%) per cross [3]					-12.15 to 14.21		-30.57 to 21.83		-37.26 to 26.25		-35.19 to 41.03		35.35 to -33.58		0.49 to -0.25	

[1] Estimates obtained with three replications.

[2] MD : PHM: Correlated response in PHM caused by selection on MD (i = 10%).

[3] Among 30 plots (10 experimental lines x 3 replications) of each cross.

for MD, and between 35.35% and -33.58% for SY. While selecting for seed yield (SY), some crosses showed higher Rs for MD resistance: between -35.19% and 41.03% for MD, and between 0.49% and -0.25% for PHM.

MD genotype for early cycle soybean is considered promising for obtaining inbred lines more resistant to MD. The choice of parents and method of handling experimental work both in the laboratory and in the field are very important, once they can reduce segregant population variability (when similar parents are chosen) and increase experimental variation values.

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RESUMO

Análise Genética da Reação da Semente de Soja ao Dano Mecânico

A suscetibilidade da semente de soja (*Glycine max* (L.) Merrill) ao dano mecânico durante os procedimentos de colheita, debulha e armazenamento tem diminuido a qualidade da semente, pois reduz a capacidade de germinação da semente e o vigor da plântula, principalmente em ambientes tropicais e subtropicais. Esta pesquisa objetivou a estimação da variabilidade genética da resposta da semente ao dano mecânico (MD) e suas relações com caracteres agronômicos, tais como altura da planta na maturidade (PHM) e produtividade de grãos (SY). O material compreendeu dez parentais precoces, os 45 cruzamentos dialélicos entre eles e cinco testemunhas. Cada cruzamento foi representado por dez linhagens experimentais. As análises estatístico-genéticas incluiram estimativas de correlações fenotípica e genotípica; contribuição relativa de efeitos genéticos *versus* a combinação de efeitos genéticos e ambientais; quociente entre os coeficientes de variação genético e experimental; coeficiente de determinação genotípica; ganhos genéticos esperado e observado na seleção; e, resposta correlacionada à seleção. Os parentais Paraná, Hale-321, IAC-Foscarin-31 e Primavera mostraram o mesmo nível de resistência que o cultivar IAS-5, uma testemunha padrão de resistência ao MD. Já os parentais Palmetto, Coker-136, FT-81-1835 e Forrest foram suscetíveis ao MD; entretanto, eles mostraram-se superiores ao cultivar IAC-2, uma testemunha padrão de suscetibilidade ao MD. O coeficiente de determinação genotípica para MD foi estimado em H = 0,739, indicando alta probabilidade de se selecionar linhagens resistentes ao MD na população representada pelos 45 cruzamentos dialélicos. Os ganhos genéticos esperado e observado na seleção também indicaram a possibilidade de sucesso na seleção para resistência ao MD, entre e dentro dos cruzamentos. Além disso, devido apresentarem baixos valores ($< 0,25$) de correlação genotípica, os caracteres MD, PHM e SY podem ser reunidos em uma mesma planta, em todas as combinações favoráveis.

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