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Estimates of repeatability in the evaluation of resistance of soybean genotypes to powdery mildew

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ABSTRACT - *Soybean is worldwide a crop of great importance, however, with its expansion, diseases causing great yield loss such as the powdery mildew surge (*Erisyphe diffusa *U. Braun & S. Takam). Objective of the present study was to estimate the ideal number of evaluations of the resistance of soybean genotypes to powdery mildew by means of repeatability studies with the traits: ILA (infection of the leaf area), ILS (infection of the leaf blade surface) and ILU (infection of the leaf underside), using the treatments no control - NC, partial control - PC (fungicide 80% sulphur at ILA $\geq$ 40%) and total control - TC (presence of symptoms). Only trait ILA presented estimates of repeatability (ANOVA and principal components), with 85-90% of stability in 3 or 4 measurements (PC and NC methods). These results and the high values found for R^2 indicate that selection based on this parameter is possible.*

Key-words: repeatability, powdery mildew, soybean, phenotypic variance.

INTRODUCTION

Soybean (*Glycine Max* L. Merrill), is one of the legumes that most raise expectations in all the world, due to the commercial importance of its products and subproducts, actually used by the food agroindustry and by the chemical industry (Embrapa 2002). Brazil, the world's second-largest producer, came up for 50 of the 200 million tons produced at global level in 2004 (Embrapa 2005).

Throughout the crop cycle, soybean can suffer under the action of some 40 diseases provoked by fungal, bacterial, viral and nematode microorganisms (Embrapa 1998). Among the diseases that befall soybean, powdery mildew caused by the fungus *Erisyphe diffusa* U. Braun & S. Takam has become well-known in the last six years as a disease of great economical impact (exceeding levels of 30% of the production), regardless of the use of crop rotation. We emphasize that the infection caused by the *E. diffusa* can occur at any development stage of the plant and that the earlier it sets in, the greater will be the effect of the disease on the plant yield (Embrapa 1998). Chemical treatments with recommended fungicides can be used for the control of powdery mildew, yet not until the level of the infection reaches 40 to 50% of the leaf area. The most efficient control of powdery mildew is however the use of resistant cultivars (Embrapa 1998).

Studies on genetic heritability by Buzzell and Haas (1978) confirmed the results Grau and Laurence (1975)

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found for the segregation of three resistant adult soybean plants (Chippewa 64) to a susceptible (Corsoy), indicating the existence of a single dominant gene which they called *Rmd* (resistant) and *rmd* (susceptible). However, the same authors report that a second gene may also be involved in powdery mildew-resistance. Studies by Lohnes and Bernard (1992) and Lohnes and Nickell (1994) into the resistance of soybean cultivars also show the ratio 3:1, confirming the hypothesis of the expression of a second gene.

Repeatability studies are of great importance in genetic improvement studies since once a certain genotype is selected for its performance or integrant structures of the same, it is expected that this performance lasts throughout the entire life cycle. The veracity of this expectation can be confirmed by the repeatability coefficient of the study trait, which can be estimated when a trait is measured on a same individual, repeatedly, in time or space. From the statistical point of view the correlation between the referred repeated measurements is known as repeatability (Lush 1945, Turner and Young 1969, Lerner 1977, Cruz and Regazzi 1994). Thus, it represents the proportion of the total phenotypic variance of a trait that is explained by permanent differences among individuals (Chapman 1985).

The repeatability coefficient consists in a measurement of the expected gain of multiple measurements, in the precision of the inference on the genotypic value of the individual. When the number of measurements increases, the variance caused by the temporal environment diminishes and the phenotypic variance is, consequently, reduced. This reduction of the phenotypic variance represents the gain in precision (Falconer 1989).

Different methods are available to estimate the repeatability coefficient, among which the analysis of variance and of the principal components can be cited. Our study had the objective of estimating the ideal number of evaluations of powdery mildew-resistance in soybean genotypes to by means of repeatability studies.

MATERIAL AND METHODS

The experiment was installed and conducted in the period of Oct./Dec. of 1998 in a greenhouse at the Department of Plant Science of the Universidade Federal de Viçosa-UFV, Viçosa, MG, Brasil. The temperature and relative humidity inside the greenhouse oscillated from 14.6 to 22.9 °C and 33.7 the 95.5%, respectively. The completely randomized block design arranged in split plots was used, with 15 treatments (genotypes), three control types representing the plots and five evaluations (subplots). The experimental material consisted of the varieties UFV-16 - Capinópolis (resistance standard), UFV-19 (Triângulo), FT-104, Doko RC, FT-Cristalina, FT-Estrela (susceptibility standard), and BR-16; and the lines UFV 89-361826t2, FT-Abyara RC_5 (F_4), FT-Abyara RC_6 (F_2), FT-10 RC_5 (F_3), UFV 94-5126, UFV 94-3500, UFV 95-4121333, and UFV 94-334268, obtained in the crop year of 1999.

To determine the reaction of soybean genotypes to powdery mildew in different evaluation periods, seeds treated with the fungicide tetramethylthiuram disulfide (TETD) were sown directly in pots (3 liters) filled with a substrate composed of a homogenized mixture of soil and dung under daily irrigation until sowing. Eight to ten seeds per pot were distributed (30.10.98) and just before the moment of inoculation, the plants were thinned out, leaving only 4 plants per pot.

The seedlings were inoculated by means of V_1 plants of the soybean variety FT-Estrela (susceptible) infected with the causal fungus of powdery mildew (Fehr et al. 1971, Costa and Marchezan 1982), between the 16th and 20th day after sowing, placing the pots with powdery mildew-infected plants alternating with pots containing the experimental material. After inoculation, the plants remained in a greenhouse under irrigation and topdressing fertilization with ammonium sulphate, whenever necessary.

Three strategies were applied in this research, which were the different control levels: (a) without control – NC (without 80% sulphur), (b) partial control– PC (application of 80%sulphur when ILA $\geq$ 40%) and (c) total control – TC (application of 80% sulphur whenever symptoms appeared). The fungicide dosage was quantified in 1.5 g/liter and the PC was realized from the second evaluation on. The following traits were evaluated: infection of the leaf area (ILA), infection of the leaf blade surface (ILS) and infection of the leaf underside (ILU).

The incidence and severity of powdery mildew was evaluated by means of a visual quantification of the infection level (IL), by which ILA, ILS and ILU were evaluated according to the adapted scales proposed by Lohnes and Nickell (1994) and Yorinori (1997). The scores varied from 1.0 to 5.0, applying the following criteria: IL=1.0 (plants without symptoms or visible signs), IL=2.0 (1 to 25% infected leaf area– ILA), IL=3.0 (26 to 50% ILA), IL=4.0 (51 to 75% ILA) and IL=5.0 (>75% ILA) in 5 evaluations with intervals of seven days after the plantlet inoculation.

The statistical and genetic analyses were done by means of analysis of variance and repeatability estimates, using software Genes (Cruz 1997). The repeatability estimates were realized by the methods of analysis of variance and of the principal components obtained from the matrixes of covariances and correlations, according to the following model:

$$Y_{ij} = \mu + g_i + \varepsilon_{ij}$$

where

Y_{ij} :observation referring to the ith genotype in the jth period

μ: general mean

g_i :random effect of the ith genotype under the influence of the permanent period (i=1,2,3,...p)

ε_{ij} : effect of the temporal period associated with the jth evaluation in the ith genotype

(j= 1, 2,..., n_i)

The repeatability coefficient was obtained by means of the correlation among the measurements repeated in a same individual; these evaluations were repeated in time and space according to the formula below:

$$r = \hat{\rho} = \frac{\hat{Cov}(Y_{ij}, Y_{ij})}{\sqrt{\hat{V}(Y_{ij})\,\hat{V}(Y_{ij,})}} = \frac{\hat{\sigma}_g^2}{\hat{\sigma}_g^2} = \frac{\hat{\sigma}_g^2}{\hat{\sigma}^2 + \hat{\sigma}_g^2}$$

where

Y_{ij} and $Y_{ij'}$: are the different measurements of a same individual, in different periods or times

$\hat{\sigma}_g^2$: estimator of the permanent environment confounded with the component of genetic variance

$\hat{\sigma}_y^2$: estimator of the temporal environment.

RESULTS AND DISCUSSION

Analysis of variance

The results of the analysis of variance for the traits ILA, ILS and ILU when powdery mildew was not controlled, partially or totally controlled are displayed in the Table 1.

These results are significant at the level of 5% probability by the F test for the genotypes and periods for all traits, indicating the existence of different performance the genotypes in relation to the studied traits and to the periods of evaluation. According to Gravina et al. (2004), the significance stated in the analysis of variance indicates the existence of additional and non-additional genetic variability among the evaluated genotypes. On the other hand, the coefficient of variation values between 8.42 and 27.41% in the analysis signalized a good precision of the experiment (optimal to regular).

Estimates of the number of evaluations needed for certain coefficients of determination

The analysis of variance is an alternative way of obtaining the repeatability coefficient, in which the temporal environment effect is removed from the error, or the temporal effect of the environment is confounded with the error. The repeatability coefficient is very useful in the possibility of determining how many phenotypic observations must be realized in each individual so that the phenotypic selection among the genotypes is effective and requires a minimum of cost and labor.

The results regarding the number of evaluations needed to characterize the resistance level of soybean genotypes to powdery mildew are presented in the Tables 2, 3 and 4 for the traits ILA, ILS and ILU, using the methods without control, partial and total control, respectively.

Trait ILA alone presented 85 to 90% of genotypic stability in only 3 or 4 evaluations realized with the estimators of ANOVA, as much as with the estimators of the principal components (Table 2). According to Carvalho and Cruz (2003) and Falconer (1989), this happens because the variation within genotype measurements is not only caused by the temporal environment, but also by the additional variation brought about by the interaction genotype and environment. This additional variance can be sufficient to neutralize the reduction of the variance caused by the temporal environment and, consequently, neutralize the increase in the prevision of the influence on the real value of the genotype, which consists in the principal

Table 1. Summary of the analysis of variance of the infection of the leaf area (ILA), infection of the leaf surface (ILS) and infection of the leaf underside (ILU) evaluated during five periods in 15 soybean genotypes in relation to powdery mildew using the treatments no control - NC, partial control - PC and total control - TC control of the disease under greenhouse conditions

Sources of Variation	df	MS (NC)			MS (PC)			MS (TC)		
		ILA	ILS	ILU	ILA	ILS	ILU	ILA	ILS	ILU
Genotypes	14	2.11*	0.81*	0.97*	2.13*	2.57*	2.54*	0.10*	0.52*	0.36*
Periods	4	2.82*	2.03*	10.52*	2.77*	2.11*	4.21*	0.76*	5.69*	5.15*
Error	56	0.15	0.14	0.34	0.17	0.23	0.34	0.03	0.21	0.21
Total	74	5.08	2.98	11.83	5.07	4.91	7.09	0.89	6.42	5.72
Mean		3.43	4.45	3.99	2.84	4.13	3.82	1.45	1.92	1.67
CV (%)		11.41	8.42	14.60	14.52	11.57	15.26	12.67	23.85	27.41

*Significant at 5% probability by the F test

Table 2. Estimates of the number of evaluations needed for certain coefficients of determination (R^2) for the traits: infection of the leaf area (ILA); infection of the leaf surface (ILS) and infection of the leaf underside (ILU) for 15 soybean genotypes in relation to powdery mildew with no control (without fungicide application) under greenhouse conditions

R^2	ILA		ILS		ILU	
	ANOVA Comp.	(corr.)	ANOVA Comp.	(corr.)	ANOVA Comp.	(corr.)
80.00	2	2	5	3	11	10
85.00	3	3	6	5	16	13
90.00	4	4	10	7	25	21
95.00	8	7	20	14	51	44
99.00	39	36	104	73	266	227

Table 3. Estimates of the number of evaluations needed for certain determination coefficients (R^2) for the traits: infection of the leaf area (ILA); infection of the leaf surface (ILS) and infection of the leaf underside (ILU) for 15 soybean genotypes in relation to powdery mildew under partial disease control (fungicide application when ILA $\geq$ 40%) under greenhouse conditions

R^2	ILA		ILS		ILU	
	ANOVA Comp.	(corr.)	ANOVA Comp.	(corr.)	ANOVA Comp.	(corr.)
80.00	2	2	2	2	4	3
85.00	3	3	3	3	5	5
90.00	4	4	5	4	7	7
95.00	9	8	10	9	15	14
99.00	43	41	49	44	77	72

Table 4. Estimates of the number of evaluations needed for certain coefficients of determination (R^2) for the traits: infection of the leaf area (ILA); infection of the leaf blade surface (ILS) and infection of the leaf underside (ILU) for 15 soybean genotypes in relation to powdery mildew under total disease control (fungicide application at the appearance of powdery mildew symptoms) under greenhouse conditions

R^2	ILA		ILS		ILU	
	ANOVA Comp.	(corr.)	ANOVA Comp.	(corr.)	ANOVA Comp.	(corr.)
80.00	10	9	14	9	29	19
85.00	14	12	20	12	40	26
90.00	22	19	31	19	64	41
95.00	46	39	65	40	134	86
99.00	238	202	334	209	696	446

advantage of having been obtained by multiple measurements (Falconer 1989). Additional genic effects were also observed in studies realized by Fronza et al. (2004).

For the traits ILS and ILU the estimate of repeatability was low, which can be explained by the interaction of genotypes x periods and by the higher coefficient of variation (Table 1). This is the reason for the need of a larger number of measurements to attain 85 to 90% of certainness of the real selected genotypic value.

In Table 3, the trait ILA presented the same determination level as above in the three or four evaluations carried out by the two estimator methods, indicating that there was optimum regularity in the repetition of the genotype performance in relation to the studied trait from one measurement to another, while for ILS three to five evaluations were needed for both applied estimator methods and for the trait ILU five to seven measurements. These two last traits showed better genotypic regularity under the partial disease control, compared with the results obtained in Table 2.

For a genotype discrimination with a 85 to 90% reliability of evaluating the real value of each one however, 14 to 64 measurements were needed with the estimators of ANOVA and 12 to 41 measurements with the estimators of the components of correlation, respectively, for all studied traits (Table 4). In this case it could be suggested that the value of stability of the genotypes is relatively low, so many measurements were necessary to evaluate the relative superiority of one genotype over the other, and such an increase in the number of measurements is undesirable in terms of time and labor savings.

Analysis of repeatability (ρ) and of the coefficient of determination (R^2)

The methods ANOVA and principal components extracted from the matrixes of covariance and correlation led to different estimates of the repeatability coefficient. The results of the estimates of the coefficients of repeatability and of determination for the traits ILA, ILS and ILU (methods without control and partial control) are presented in the Table 5.

In method without control, trait ILA attained the highest value for the repeatability coefficient in all estimators of ANOVA and of the principal components of the matrix of covariance and phenotypic correlation. However, the two latter estimators presented even higher values than those of the analysis of variance, expressing expressive differences in the performance of the different genotypes. Nevertheless, it can be suggested that there was regularity in the repetition of the genotype performance of one evaluation to the other, evidencing that the selection based on the parameter of estimates of repeatability can be effective (Farias et al. 1998, Leão and Costa 2003).

The results of Table 5 show that in partial control disease, there was values close to those achieved by the same trait ILA in method without control, suggesting a tendency of the genotypes to maintain their relative positions in relation to the others during the period of evaluation. However, trait ILS also presented significant and similar values to those of trait ILA as well as trait ILU, although the values of this one (method without control) were of low magnitude for the estimators of ANOVA, as much as for those of the principal components, indicating a poor genotypic performance of this last trait.

Table 6 shows that all traits ILA, ILS and ILU presented repeatability estimates of lower magnitude found by the estimators of ANOVA and of the principal components, despite considered expressive, with values of 0.57 and 0.51, respectively, for ILA and ILU. Suggesting that by this method there was a considerable similarity in the repetition of the genotypic performance of one evaluation with another of these traits, however, when estimated by the principal components obtained of the matrix of covariance.

The lowest ρ value was achieved by the method of analysis of variance (ANOVA) with the environment effect removed from the error, while the highest ñ value was achieved by the method of the principal components obtained from the matrix of covariance and of correlation (Tables 5 and 6), which can suggest the existence of expressive differences in the performance of the different genotypes.

In respect to the coefficient of determination (R^2), which evaluates the predictability or stability of the material (Farias et al. 1998), the estimates of repeatability regarding R^2 were of high magnitudes, principally for trait ILA, varying from 92.75 without control to 93.00% with partial control by fungicide application. Trait ILU however presented R^2 of 41.60% (Table 6), considered of low magnitude in relation to the values shown above.

Although the values of ρ are of low magnitude for some traits (Tables 5 and 6), a good accuracy is observed for the prediction of the real value of the individuals, estimated by the coefficient of determination (R^2). It can therefore be inferred that the genotypic selection based on values considered of high magnitude, in respect to the stability in function of the R^2, can be efficient.

CONCLUSIONS

The conclusion was drawn that the ideal number of measurements required to estimate the resistance level of soybean genotypes to powdery mildew is three to four. Regarding the estimates of repeatability, using the estimators of ANOVA and of the principal components obtained through the matrix of covariance and of correlation, the values considered of high magnitude of the repeatability coefficient presented by the trait ILA in the methods without control and partial control, show the efficiency of this trait for the indication of resistant genotypes, as well as the importance of the study of repeatability for selection goals of resistant material of soybean to powdery mildew. The high values found for R^2 further evidenced that the selection based on this parameter is possible.

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Table 5. Estimates of the coefficients of repeatability (ρ) and of determination (R^2) for the traits: infection of the leaf area (ILA); infection of the leaf blade surface (ILS) and infection of the leaf underside (ILU) for 15 soybean genotypes in relation to powdery mildew using the treatments no control - NC and partial control - PC control of the disease under greenhouse conditions

METHOD	NC						PC					
	ILA		ILS		ILU		ILA		ILS		ILU	
	ñ	R^2 (%)	ñ	R^2 (%)	ñ	R^2 (%)	ñ	R^2 (%)	ñ	R^2 (%)	ñ	R^2 (%)
ANOVA	0.7190	92.75	0.4890	82.71	0.2720	65.10	0.6799	92.03	0.6720	91.10	0.5644	86.63
Covariance	0.7300	93.10	0.5445	85.70	0.3640	74.10	0.7300	93.00	0.6874	91.70	0.5982	88.20
Correlation	0.7400	93.40	0.5800	87.30	0.3045	68.64	0.7101	92.45	0.6942	91.90	0.5800	87.40

Table 6. Estimates of the coefficients of repeatability (ρ) and of determination (R^2) for the traits: infection of the leaf area (ILA); infection of the leaf blade surface (ILS) and infection of the leaf underside (ILU) for 15 soybean genotypes in relation to powdery mildew using the treatment total control - TC control of the disease under greenhouse conditions

METHOD	ILA		ILS		ILU	
	ñ	R^2 (%)	ñ	R^2 (%)	ñ	R^2 (%)
ANOVA	0.2941	67.60	0.2300	59.74	0.1246	41.60
Covariance	0.5700	86.83	0.4700	81.60	0.5100	83.70
Correlation	0.3280	70.94	0.3222	70.40	0.1820	52.61

Estimativas de repetibilidade na avaliação da resistência de genótipos de soja ao oídio

RESUMO - *A cultura da soja é de grande importância no mundo, porém, com sua expansão, surgem doenças responsáveis por grandes perdas na produção, como o oídio (*Erisyphe diffusa *U. Braun & S. Takam). O objetivo do presente trabalho, foi estimar o número ideal de avaliações da resistência de genótipos de soja ao oídio, por meio de estudos de repetibilidade, com as características IAF (infecção da área foliar), IFS (infecção da face superior do folíolo) e IFI (infecção da face inferior do folíolo), usando os tratamentos sem controle - SC, controle parcial - CP (fungicida enxofre 80%, IAF $\geq$ 40%) e controle total*

- CT (presença de sintomas). Só a característica IAF apresentou estimativas de repetibilidade (ANOVA e componentes principais), com 85-90% de estabilidade em 3 ou 4 medições (métodos CP e SC). Estes resultados e os altos valores encontrados para o R^2, indicam que a seleção baseada neste parâmetro é possível.

Palavras-chave: repetibilidade, oídio, soja, variância fenotípica.

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