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NOTE

Reaction of soybean lines to stem canker

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ABSTRACT - *Objectives of this study were the evaluation of the reaction to stem canker in F_8 soybean lines; analysis of the consistence of the symptom evaluation in different periods; as well as the consistence of scores based on different numbers of plants per row. Twelve days after sowing the plants were inoculated with isolate CH 08 by the tooth-toothpick technique and the symptoms evaluated 10, 20, 40, and 73 days after the inoculation (DAI). Ten lines with resistance reaction were identified. The scores attributed in the different evaluation periods were in line and the evaluations realized 10 and 20 DAI were sufficient for an identification of lines with resistance reaction. Five plants per treatment are sufficient for an evaluation of the reaction in endogamic lines.*

Key words: *Diaporthe phaseolorum*, *Glycine max*, genetic resistance.

INTRODUCTION

Until the harvest of 1996/97, the accumulated loss through stem canker caused by *Diaporthe phaseolorum* f. sp. *meridionalis* (Embrapa 2002) was an estimated US$ 0.5 billion. The damage this disease caused, along with its dissemination potential and impact on the yield made the launching of canker-susceptible diseases cultivars unfeasible. Studies on heritability have shown that the resistance, whose transference is relatively simple, is controlled by dominant alleles in one (Almeida and Kiihl 1998), two (Kilen et al. 1985, Kilen and Hartwig 1987, Bowers et al. 1993) or three loci (Azevedo et al. 2001).

The reaction of genotypes to stem canker is determined principally by the inoculation method with an infected toothpick. This technique has proved adequate, since genotypes with resistance reaction towards artificial inoculation are field-resistant as well. In fact, some genotypes present susceptibility reaction to inoculation by the toothpick method and resistance reaction under field conditions. It is believed that the technique of the infected toothpick is very aggressive, overcoming certain defense mechanisms that are effective against natural field infections. In this sense, the toothpick method is recommended in the EUA for the evaluation of genotypes whose resistance source is Tracy M, while for genotypes whose resistance is derived from Centennial, the reaction should be evaluated under field conditions (Backman et

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al. 1985, Weaver et al. 1988).

Our study had the following objectives: investigation of the reaction of some F_8 lines to stem canker, observation of the consistence of the visual scores throughout the evaluation periods and evaluation of the concordance between scores based on different number of plants per row.

MATERIAL AND METHODS

The present study was conducted as part of the Genetic Soybean Improvement Program of the Department of Plant Science, at the Universidade Federal de Viçosa. The experiments were conducted in a greenhouse from 12/12/01 to 07/03/02. Twelve F_8 lines were evaluated in the first experiment, six derived from a cross of Coker 6738 x FT Cristalina $RC4F_4$ (population IV) and the other six from Agratech 550 x FT Cristalina $RC4F_4$ (population V), the parents and three standards, BR 16 (standard of susceptibility), UFV 19 and CAC 1 (c). The experimental design was completely randomized with four replications wherein every experimental unit consisted of a pot with five plants. The second experiment evaluated six F_8 lines, selected from a crossing of CEPS 8926 x IAC 8 (population II) and the cultivars BR 16, CAC 1 and IAC 8. The completely randomized design was used with five replications. Each replication consisted of only one plant. Besides the regular treatments, replications of the cultivars BR 16 and CAC 1, which were not inoculated, were added as reference in the visual vigor evaluation.

The plants were inoculated 12 days after sowing by introducing toothpicks colonized with the fungus mycelium (isolate CH 08) into the main plant stem, right under the node of the unifoliate leaves (Yorinori 1994). For the ten days following inoculation, the plants were intermittently irrigated by a spraying system.

The symptom intensity of stem canker was evaluated by scores attributed visually to the lesion size (1- absence of lesion; 2- up to 1.0 cm long lesion in the main stem; 3- 2.0 to 2.9 cm long lesion in the main stem; 4- 3.0 to 3.9 cm long lesion in the main stem; and 5- over 4.0 cm long lesions in the main stem) and for plant vigor (1- normal plant; 2- curving of the apical shoot or slight reduction in the growth of the leaf nervure; 3- symptom of beginning leaf lesion above the inoculated node; 4- necrosed leaf with beginning lesion of the meristem or death of the upper leaves or wilt; and 5- death of the apical meristem. Evaluations were realized 10, 20, 40, and 73 days after inoculation (DAI). Based on the last evaluation and according to the criterion proposed by Weaver et al. (1988), the reaction of the genotypes was classified as: R = resistant (score = 1.0); MR = moderately resistant (score between 1.1 and 2.0); MS = moderately susceptible (score between 2.1 and 3.0); S = susceptible (score between 3.1 and 4.0); or HS = highly susceptible (score > 4.1).

Correlations between the lesion and vigor scores in the four periods of evaluation were estimated in order to analyze the consistence of the scores given to the treatments in different periods. In Experiment 1, the correlations between the scores attributed to the replication and to the mean scores of the treatments (four replications) were estimated to analyze the association between the scores attributed to the treatments considering different numbers of plants. In the evaluation of the coefficient of correlation between the replications and the mean of the treatments (four replications) one should bear in mind that the replication was also taken into consideration to obtain the mean, so the two variables are not independent. Consequently, even if the covariances between replications are zero, the coefficient of correlation is 0.5 when the variance of the replications is equal. One should therefore consider coefficients of correlation near 0.5 as of low magnitude.

RESULTS AND DISCUSSION

The lines of population IV (Coker 6738 x FT Cristalina $BC4F_4$) varied in performance in the first evaluation (10 days after inoculation), while the lines of population V (Agratech 550 x FT Cristalina $BC4F_4$) showed no symptoms (Table 1). Stem canker symptoms were observed in cultivar Coker 6738, opposite to parent Agratech 550. The common parent of both populations, FT Cristalina BC4, received score 1.10 for lesion size and for vigor. In the subsequent evaluations however, no disease symptoms were observed, confirming the incorporation of genes of stem canker resistance. The stem canker-resistant cultivars UFV 19 and CAC 1 were symptom-free. Cultivar BR 16 on the other hand presented lesions in the stems (score 2.60) and, simultaneously, alterations in growth, graded with score 2.85 for plant vigor, from the first evaluation on (Table 1). In the evaluations realized 20 and 40 DAI there were few alterations in relation to the first evaluation, but an increased symptom intensity was observed in the genotypes with some susceptibility degree. P.IV-2 and P.IV-7 stood out in the evaluation realized 40 DAI, principally

Table 1. Means of the scores of lesion extension and plant vigor 10, 20, 40, and 73 days after the inoculation (DAI) with *Diaporthe phaseolorum* f. sp. *meridionalis* in soybean lines of the populations IV and V and the respective standards (Experiment 1)

Treatment	10 DAI		20 DAI		40 DAI		73 DAI		
	Lesion	Vigor	Lesion	Vigor	Lesion	Vigor	Lesion	Vigor	Reaction (73 DAI)
P.IV-1[1]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	Resistant
P.IV-2	2.00	1.85	1.95	2.30	2.30	2.45	2.85	2.85	Moderately Susceptible
P.IV-6	1.00	1.00	1.00	1.05	1.00	1.05	1.00	1.00	Resistant
P.IV-7	1.60	1.65	1.75	1.95	2.10	2.15	3.09	2.86	Susceptible
P.IV-8	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	Resistant
P.IV-15	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	Resistant
Coker 6738	1.95	1.85	2.80	2.70	2.85	2.95	3.30	3.35	Susceptible
FT-Cris.BC4[2]	1.10	1.10	1.00	1.00	1.00	1.00	1.00	1.00	Resistant
Agratech 550	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	Resistant
P.V-5[3]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	Resistant
P.V-7	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	Resistant
P.V-12	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	Resistant
P.V-13	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	Resistant
P.V-14	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	Resistant
P.V-15	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	Resistant
UFV 19	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	Resistant
CAC 1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	Resistant
BR 16	2.60	2.85	4.40	4.05	4.85	4.85	5.00	5.00	Highly Susceptible
CAC 1 s/I[4]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	–
BR 16 s/i	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	–

[1] P.IV = population IV – Coker 6738 x FT Cristalina $BC4F_4$
[2] FT-Cris.RC4 = FT Cristalina BC4
[3] P.V = population V – Agratech 550 x FT Cristalina $BC4F_4$
[4] s/i = no inoculation

with a reaction of moderate susceptibility. Coker 6738 and BR 16 also presented more intense stem canker symptoms, especially in BR 16, in which the scores attributed to lesions and vigor were 4.85, expressing high susceptibility (Table 1). The last evaluation (73 DAI) showed further developed symptoms of the treatments that had shown some susceptibility degree in the previous evaluations. Among the 12 evaluated lines only P.IV-2 and P.IV-7 did not present resistance reaction. Cultivar Coker 6738 is recommended as stem canker-resistant (Clemson University 1993; Clemson University 1997) in the United States. It in fact presented a susceptibility reaction in this experiment when inoculated with isolate CH 08. This result is probably a consequence of the pathogenic variability among the *D. phaseolorum* f. sp. *meridionalis* isolates used in the evaluations.

Cultivar BR 16 is typically susceptible when inoculated by the toothpick method (greenhouse) and, simultaneously presents moderate field resistance to the same disease (Fepagro 2001, Embrapa 2002). Results of this experiment confirmed the susceptibility of BR 16 under inoculation (Table 1). The North-American cultivars Centennial and Peking also presented a susceptibility reaction towards inoculation (toothpick method) and resistance against natural infections by the causal agent of stem canker in the northern region of the United States (*D. phaseolorum* f. sp. *Caulivora*). It is

assumed that cultivars with field resistance carry distinct alleles from those present in the other cultivars (Backman et al. 1985, Weaver et al. 1988).

Ten days after inoculation the lines of population II were graded with scores that expressed susceptibility. Cultivars IAC 8 and BR 16 presented a moderate susceptibility reaction, opposite to CAC 1. The evaluations realized 20 and 40 DAI demonstrated a progress of the symptoms in genotypes with some degree of susceptibility, in the lesion size as much as in a reduced plant vigor. Cultivar CAC 1, evidenced in Experiment 1 as stem canker-resistant, did not present any symptom with the inoculation. The reactions of genotypes 20 and 73 DAI were observed to be highly concordant (Table 2). All lines of population II presented high susceptibility to stem canker (73 DAI). All plants of the lines P.II-4, P.II-7, P.II-13, and P.II-15 were dead at the point of this last evaluation (Table 2). Parent IAC 8 reacted with susceptibility, while the standards BR 16 and CAC 1 presented, respectively, reaction of high susceptibility and resistance, repeating the result of the first experiment.

The coefficients of correlation estimated between the evaluations of lesion size and plant vigor of the four periods of evaluation were higher than 0.95 in the first and higher than 0.74 in the second experiment. The magnitude of the coefficients of correlation between lesion and vigor in the same evaluation indicated a positive association between the scores attributed to the two considered variables. The magnitude of the correlations evidences the concordance between evaluations realized in the four periods. The scores presented in the Tables 1 and 2 express this fact likewise. One should however consider that the high correlations did not necessarily indicate a possibility of reducing the number of evaluations. The correlation is a measure of association or of how much two variables vary jointly, which in the present case indicates that there is a strong tendency of constant or increasing scores in the treatments. The classification of the reaction 10 and 73 DAI would be coincident for the resistant treatments, but not for those that presented some susceptibility degree. On the other hand, the treatments with higher scores maintained or presented increment in the levels of symptoms. Also in relation to the evaluation 20 and 40 DAI, the correlations indicated a strong association between the attributed scores. In experiment 2, the classification of the reaction to canker after 40 and 73 days was totally coincident (Tables 2 and 3). These results indicate the viability of the visual evaluation, since the scores given to the treatments in the different periods of evaluation were consistent. One can further state that in the present case, the two first evaluations would be sufficient to select genotypes with resistance reaction. To discriminate genotypes with different levels of susceptibility posterior evaluations are necessary, as for example the evaluation 40 DAI. This observation is in line with the results presented by Weaver et al. (1988).

Table 2. Means of the scores of lesion extension and plant vigor 10, 20, 40, and 73 days after inoculation (DAI) with *Diaporthe phaseolorum* f. sp. *meridionalis* in soybean lines of the population II (CEPS 8926 x IAC 8) and in the respective standards (Experiment 2)

Treatment	10 DAI		20 DAI		40 DAI		73 DAI		Reaction (73 DAI)
	Lesion	Vigor	Lesion	Vigor	Lesion	Vigor	Lesion	Vigor	
P.II-4	3.20	2.80	4.60	4.20	5.00	5.00	5.00	5.00	Highly Susceptible
P.II-7	2.40	2.60	4.20	3.80	5.00	5.00	5.00	5.00	Highly Susceptible
P.II-10	1.60	2.20	2.80	2.80	4.20	4.20	4.20	4.20	Highly Susceptible
P.II-12	2.40	3.00	4.00	3.60	4.20	4.40	4.20	4.20	Highly Susceptible
P.II-13	2.80	3.40	5.00	3.80	5.00	5.00	5.00	5.00	Highly Susceptible
P.II-15	3.60	3.80	5.00	5.00	5.00	5.00	5.00	5.00	Highly Susceptible
IAC 8	2.80	2.60	3.40	3.20	3.40	3.40	3.40	3.40	Susceptible
BR 16	2.60	2.60	5.00	4.00	5.00	5.00	5.00	5.00	Highly Susceptible
CAC 1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	Resistant
BR 16 n/i[1]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	–
CAC 1 n/i	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	–

[1]n/i = no inoculation

Table 3. Estimates of the coefficients of phenotypic correlation between the evaluations of extension of lesion and plant vigor realized 10, 20, 40, and 73 days after the inoculation (DAI) with *Diaporthe phaseolorum* f. sp. *meridionalis* in Experiment 1 (above) and in Experiment 2 (below diagonal)

	Observations[1]							
Observations	**L10**	**V10**	**L20**	**V20**	**L40**	**V40**	**L73**	**V73**
L10	- -	0.99	0.96	0.99	0.98	0.98	0.98	0.99
V10	0.89	- -	0.98	0.99	0.99	0.99	0.99	0.99
L20	0.87	0.89	- -	0.99	1	0.99	0.96	0.97
V20	0.93	0.92	0.95	- -	1	1	0.99	0.99
L40	0.75	0.82	0.93	0.92	- -	1	0.98	0.99
V40	0.74	0.83	0.93	0.92	1	- -	0.98	0.99
L73	0.75	0.82	0.93	0.92	1	1	- -	1
V73	0.75	0.82	0.93	0.92	1	1	1	- -

Four replications were used in Experiment 1, each one composed of five plants - a number above the commonly used in investigations of reaction to stem canker. The studies of Yorinori (1996) who evaluated ten plants of each genotype and Azevedo et al. (2001), who observed five plants per treatment, exemplify this aspect. To evaluate the consistence of the results considering different numbers of plants per treatment, the correlations between the scores attributed to each replication and to the means of the treatments of Experiment 1 were therefore estimated (Table 4). The lowest estimated coefficients (0.88) were between the first replication and the mean, considering the lesion size score 73 DAI and of the third replication and the mean for lesion size and plant vigor 40 DAI. As highlighted before, the coefficients of correlation near 0.5 should be interpreted as a low magnitude while in fact the correlations varied from 0.88 to 0.99, indicating a good concordance between the scores attributed to each replication and to the score mean of the four replications. Still, these conclusions were based on the evaluation of lines derived from several generations of selfing and selection so that there was little genetic variability within these progenies or lines, making the use of a lower number of plants in the evaluation feasible.

Table 4. Estimates of coefficients of correlation of the visual scores (lesion and vigor) of the replications with the score means of the treatments of Experiment 1

	Observations[1]							
Correlation[2]	**L10**	**V10**	**L20**	**V20**	**L40**	**V40**	**L73**	**V73**
R1 – Mean	0.91	0.98	0.97	0.95	0.95	0.95	0.88	0.91
R2 – Mean	0.97	0.94	0.99	0.99	0.99	0.99	0.98	0.98
R3 – Mean	0.95	0.91	0.98	0.99	0.89	0.89	0.96	0.95
R4 – Mean	0.98	0.94	0.93	0.96	0.94	0.95	0.94	0.95

Based on the observed results ten stem canker-resistant lines (P.IV-1, P.IV-6, P.IV-8 and P.IV-15, P.V-5, P.V-7, P.V-12, P.V-13, P.V-14, and P.V-15) were identified. Moreover, the conclusions were drawn that the evaluations realized in different periods after inoculation are consistent; that evaluations 10 and 20 DAI are sufficient for an identification of soybean lines with resistance reaction; and that five plants are sufficient for an evaluation of the reaction of endogamic soybean lines to stem canker.

Reação de linhagens de soja ao cancro-da-haste

RESUMO - *O objetivo deste trabalho foi avaliar a reação ao cancro-da-haste em linhagens F_8 de soja; analisar a consistência da avaliação de sintomas em diferentes épocas; e a consistência das notas com base em diferente número de plantas por linhagem. A inoculação com o isolado CH 08 foi realizada 12 dias após a semeadura, pelo método do palito, sendo as avaliações de sintomas realizadas aos 10, 20, 40 e 73 dias após a inoculação (DAI). Foram identificadas dez linhagens com reação de resistência. Houve consistência entre as notas atribuídas nas diferentes épocas de avaliação, sendo as avaliações realizadas aos 10 e 20 DAI suficientes para a identificação de linhagens com reação de resistência. Para avaliação da reação em linhagens endogâmicas, cinco plantas por tratamento são suficientes.*

Palavras-chave: *Diaporthe phaseolorum, Glycine max,* genetic resistance.

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