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An Alternative Field Method for Screening Soybean Genotypes for Resistance to *Heterodera glycines*

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Abstract: The soybean cyst nematode (*Heterodera glycines*) has become an increasingly severe problem in soybean production areas in Brazil. The development and use of resistant cultivars is the most efficient method of minimizing losses due to this pathogen. Our objective was to test the efficiency of an alternative method for screening soybean genotypes for resistance to *H. glycines* in field plots. The alternative method was compared to the standard method of sowing the test genotypes in fields found to be infested during the previous crop season. In the alternative method, the test genotypes are sown in the furrow following the uprooting of 45-day-old infected plants. The alternative method resulted in twice the cyst population and fewer escapes, and more consistent results than the standard method. The major advantage of the alternative method is that it permits screening in a more homogeneous distribution of *H. glycines* in the soil.

Key words: Heterodera glycines, resistance, screening, soybean, soybean cyst nematode.

Among cultivated plants in Brazil, soybean has had the greatest expansion in hectarage over the last few decades. Genetic improvement undoubtedly has been the main factor responsible for the success of soybean, specially in the cerrado region. Along with the increase in hectarage, many problems have arisen compromising yield and expansion of the cultivated area. Soybean cyst nematode (*Heterodera glycines* Ichinohe) is considered the most serious threat to the soybean crop in Brazil. Since its identification in 1992 (Lima et al., 1992; Lordello et al., 1992; Monteiro and Morais, 1992), the nematode has spread quickly, causing serious damage and even preventing the use of infested areas for soybean culture. Genetic improvement aimed at the development of resistant cultivars is among the most efficient and economic alternatives for reducing crop losses due to *H. glycines,* together with crop rotation using non-host species and susceptible soybean cultivars (Wrather et al., 1984).

In Brazil, evaluation of soybean genotypes for resistance to soybean cyst nematode is mostly performed in the field. The uneven distribution of cysts in the soil dictates the use of a large number of replications and repeated testing to determine whether a genotype is resistant or has escaped detection. According to Schmitt (1992), the population density of the soybean cyst nematode can be barely detectable in a given field or can vary from 10,000 to 20,000 eggs/500 cm^3 of soil among sampling points located 5 to 10 m apart. Thus, an efficient technique for evaluating soybean genotypes is essential when conducting a breeding program. To evaluate the resistance of soybean genotypes to *H. glycines,* Ross and Brim (1957) used 3-m rows with one row cultivated with a non-pubescent susceptible genotype 10 to 15 cm to the side of those being evaluated. With this procedure the pubescent genotypes could be recognized and compared to the non-pubescent and susceptible genotypes, thus increasing the confidence that genotypes free of females in the roots were resistant.

The evaluation of soybean genotypes under greenhouse conditions for resistance to *H. glycines* is the method most extensively used in the United States because of its reliability (Caviness, 1992). However, greenhouses are not always available, and the objective of the present study was to evaluate the efficiency of an alternative method for

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screening soybean genotypes for resistance to soybean cyst nematode under field conditions.

Materials and Methods

The experiment was carried out in summer 1995 on the Forquetense Farm, located in the county of Iraí de Minas, Minas Gerais State, geographical coordinates 19 °S, 49 °W, and 990-m altitude. The soil, previously covered with cerrado vegetation and with more than 8 years of cultivation, is classified as Yellow Red Latosol and was naturally infested with *H. glycines* race 3.

The genealogy and reaction to *H. glycines* race 3 of the 10 soybean genotypes used to evaluate the two screening methods are presented in Table 1. These genotypes were previously evaluated under field conditions and under greenhouse conditions to determine their reaction to *H. glycines*.

For the standard method, an infested area was chosen and demarcated during the previous year based on the presence of soybean cyst nematode signs and symptoms. Before sowing, the soil was prepared by plowing and harrowing. The rows, 0.50 meter apart, were opened with a conventional driller.

The alternative method differed from the standard one in the way the screening area was chosen. The experiment was conducted in an area sown with soybean cv. FT-Estrela in rows 0.50 m apart, 45 days previously. Plants, which were in the V_6 development stage (Fehr and Caviness, 1979) and showed signs and symptoms of *H. glycines*, were uprooted, and new rows were opened and seeds of genotypes to be evaluated were planted.

For both methods, the experimental design was a randomized block with six replications, with each plot consisting of a row 2 m long with approximately 40 plants. The areas used in this study for both standard and alternative methods were located in the same field.

In both methods, after the emergence of the plants, soil samples were collected in the first four replications to determine the initial population of *H. glycines*. Ten samples were taken from each plot at a depth of 0 to 20 cm to form a composite sample. The total number of cysts was determined in the Laboratory of Nematology of EPAMIG using the method described by Shepherd (1970).

The numbers of females and cysts on the roots were counted 34 days after sowing the soybean genotypes. In each plot, 10 plants were removed and scored according to the rating system used by Hartwig (1985) as follows: 0–no cysts on roots; 1–1 to 5 cysts; 2–6 to 10 cysts; 3–11 to 20 cysts; 4–more than 20 cysts.

In a breeding program plants scored from 2 to 4 would be considered susceptible and therefore discarded, and this parameter was used to calculate the percentage of error or escape since the reaction of the genotypes to the soybean cyst nematode was known. If, for instance, 60 plants (10 from each repli-

Table 1. Genealogy and reaction of 10 susceptible genotypes used to evaluate two methods to screen for resistance or susceptibility to *Heterodera glycines*.

Genotype	Genealogy	Reaction[a]
BR-16	D69-B10-M58 × Davis	S
Br 91-10557	Centennial[2] × BR 85-26105	R
BR 92-15266	[F81-2128 × (Kirby × Tracy M)] × Forrest	R
BR 92-15454	[F81-2128 × (Kirby × Tracy M)] × Forrest	R
BR 92-15465	Kirby × (Forrest × F81-6184)	R
CAC-1	Selection from IAC-8	S
Embrapa-20	Selection from population RB 72-1	S
FT-Cristalina	Natural cross in UFV-1	S
FT-Estrela	M-2 × FT-1	S
PF/BR 87-4291	BR84-11109 × [IAS-5[2] × Davis) × Forrest]	R

[a] S–susceptible; R–resistant.

cation) of genotype BR-16, which is susceptible to the nematode, received scores 2, 3 or 4, the percentage of error would be zero.

Analysis of variance of total numbers of cysts was performed on log (x) transformed data for both evaluation methods. Comparison of the methods was based on coefficient of variation, standard error, average standard deviation, and percentage of escape. The coefficient of variation indicates the precision of the experiment, the standard error indicates the variation among values, and the average standard deviation indicates the precision for the average estimate, with values approaching zero being more desirable in all cases (Pimentel Gomes, 1985).

The chi-square test (χ^2), as described by Pimentel Gomes (1985), was used to determine differences between the two methods. The test was used to compare the frequencies of the number of escapes (observed and expected) based on the following hypothesis: the number of escapes produced by both methods does not differ on the evaluated genotypes. On the basis of this hypothesis, tested with 4 degrees of freedom, the expected frequencies were calculated by the observed marginal product of the escape divided by the total number of escapes.

With the binomial test (Campos, 1979), it was possible to calculate the variance for the assumed binomial distribution and to verify if there were differences in the proportions of escape between the selection methods. Thus, the following variable was created for each genotype:

$X_i = 1$ if the ith plant showed escape,
0 if it did not. (1)

Since 60 plants of each genotype were evaluated with each method, the number of escapes produced for each one was obtained with the following formula (Wonnacott and Wonnacott, 1982):

$$Y = \sum_{i=1}^{60} X_i \quad (2)$$

Where Y = number of escapes.

On this basis, the proportion of escapes for each genotype was provided by the equation: $Y \cdot 60 = \sum X_i \cdot N$. With this approach, the random variable Y has binomial distribution with parameters N and P, where N = 60 is the size of the sample and P is the probability of escape occurring in the observed plant. Thus, the variance, the lowest values of which are associated with higher precision, was obtained by $N \cdot P \cdot (1 - P)$.

Results and Discussion

Numbers of cysts per 100 cm^3 of soil as determined by the two screening methods and results derived from the analysis of variance are given in Table 2. In the alternative method the coefficient of variation, standard error, and average standard deviation had lower values than those obtained by the standard method. There were no significant differences in the initial population of the soybean cyst nematode among treatments in each of the studied methods, as determined from the F test.

Average numbers of cysts in the soil (initial population) were 83 cysts/100 cm^3 soil for the standard method and 177 cysts/100 cm^3 soil for the alternative method (Table 2). Percentages of escape for both methods

Table 2. Initial number of *Heterodera glycines* per 100 cm^3 soil obtained with two screenings for *H. glycines* under field conditions and statistical parameters obtained from the analysis of variance.

Genotypes	Reaction to *H. glycines*[a]	Standard method	Alternative method
BR-16	S	89.8[b]	160.0[b]
CAC-1	S	116.5	150.5
Embrapa-20	S	69.3	162.5
FT-Cristalina	S	121.3	251.0
FT-Estrela	S	58.3	185.5
BR 91-10557	R	120.0	209.8
BR 92-15266	R	53.5	132.8
BR 92-15454	R	46.5	150.5
BR 92-15465	R	72.5	162.0
PF/BR 87-4291	R	84.5	207.8
Mean		83.2	177.3
C.V. (%)		12.54	7.22
F (treatments)		2.13NS	0.89NS
Standard error		0.5367	0.3684
Average standard deviation		0.2684	0.1844

[a] S–Susceptible; R–Resistant.
[b] Numbers are the averages of four replicates.

are presented in Table 3. Considering only the susceptible genotypes, since escape occurs when a susceptible genotype does not show cysts or females on its roots, the standard method had an error of 37.7%, which differed from 7.7% for the alternative method. This difference was significant according to the binomial test and showed that the alternative method allowed fewer escapes.

The calculated value for the chi-square test (χ^2), comparing both selection methods based on the observed and expected number of escapes, was 19.95. Since the χ^2 distribution with 4 degrees of freedom and $\propto$ = 0.05 was equal to 4.49, the hypothesis was rejected and the number of escapes obtained by the two methods was significatively different among the genotypes. The alternative method produced lower mean escape values, thus showing higher efficiency (Table 3). In all susceptible genotypes used in the present study, the variances obtained for the alternative method were lower than those obtained for the standard method (Table 3). Thus, the alternative method was more accurate and precise than the standard method.

Considering that the uneven spatial distribution of *H. glycines* is one of the difficulties to be faced when evaluating soybeans for resistance to the soybean cyst nematode in naturally infested soybean areas (Schmitt, 1992), the alternative method had specific advantages. It allowed the evaluation of an area with a higher population and with a better spatial distribution of the soybean cyst nematode population than the standard method. The higher initial population density obtained with the alternative method was due to the fact that the nematode multiplied just before sowing the test genotypes, while in the standard method the experimental area lay fallow for approximately 7 months.

The advantages of the method proposed here over that proposed by Ross and Brim (1957), in which a non-pubescent, susceptible genotype was used as control, and also over the standard method are easier operating conditions and a lower percentage of escape. Evaluation of soybean genotypes under greenhouse conditions is certainly the most reliable method (Caviness, 1992). Ideal conditions are not always available, and the alternative method for selecting soybean genotypes under field conditions represents a good option since it allows evaluation in a more homogenous cyst-infested area.

TABLE 3. Percentage of escapes of two methods for selection for resistance to *Heterodera glycines* under field conditions.

	Standard method		Alternative method	
Genotype	% escapes	Variance	% escapes	Variance
Susceptible				
BR-16	45.0	14.85	5.0	2.85
CAC-1	16.7	8.34	5.0	2.85
Embrapa-20	28.3	12.17	3.3	1.91
FT-Cristalina	43.3	14.73	25.0	5.25
FT-Estrela	55.0	14.85	0.0	0.00
Mean (S)	37.7		7.7	
Resistant				
BR 91-10557	0.0		1.7	
BR 92-15266	0.0		0.0	
BR 92-15454	0.0		0.0	
BR 92-15465	0.0		0.0	
PF/BR 87-4291	13.3		5.0	
Mean (R)	2.7		1.3	

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