# Reaction of soybean cultivars to sudden death syndrome and disease scoring methods for screening resistance*

**Luiza Helena Klingelfuss*[1]; José Tadashi Yorinori[2]; Carlos Alberto Arrabal Arias[2] and Deonisio Destro[1]**

[1]Departamento de Agronomia, Universidade Estadual de Londrina, Caixa Postal 6001, 86051-970, Londrina, PR, Brazil. [2]Embrapa Soja, Caixa Postal 231, CEP 86001-970, Londrina, PR, Brazil. (* Corresponding Authors. E-mail:lhklingelfuss@uol.com.br)

## ABSTRACT

Soybean sudden death syndrome (SDS), caused by *Fusarium solani* f. sp. *glycines,* is found in more than 2.0 million hectares of soybean fields in Brazil. Identified for the first time during the 1981/82 crop season, in São Gotardo, Minas Gerais; it had already spread to 99 counties in Central and South Brazil by the 1999/2000 crop season, causing an estimated yield loss of US$ 53 million. Since no efficient control measure is yet available , it has been carried out through resistant cultivars. One of the difficulties in selecting resistant cultivars lies on the lack of a reliable source of resistance and of a screening method to distinguish the reactions among cultivars. The objective of this study was to define a criterium for differentiating soybean cultivars reaction to SDS and to find sources of resistance to the disease, based on leaf symptoms. The study included eight soybean cultivars in four replication and were carried out in a greenhouse in two experiments. Plants were inoculated by the colonized toothpick method and assessed 21 days after inoculation , using five different assessment criteria. Results showed that during the determination of the AP % (affected plants percentile ), which revealed the different reactions to SDS among soybean cultivars, the levels of chlorosis were not significantly different from those which took into consideration the incidence of leaf chlorosis or necrosis, regardless of the severity of leaf symptoms. The %AP and the scoring method using a scale of 1 to 5 were the best procedures to assess the reaction of soybean cultivars to SDS through leaf symptoms. Cultivar FT Estrela was used as a highly susceptible standard SDS (%AP = 96.32%). Genotypes PI 567734, PI 520733 and MG/BR 46 (Conquista) were the most resistant to SDS with AP % of 30.79%, 31.30% and 35.34%, respectively. They could be used as a source of SDS resistance in crosses in breeding programs.

**KEY WORDS:** *Glycine max*, genetic resistance, *Fusarium solani*.

## INTRODUCTION

Sudden death syndrome in soybean [*Glycine max* (L.) Merrill], caused by *Fusarium solani* (Mart) Sacc. f. sp. *glycines* (Roy, 1997), was observed for the first time in Brazil at São Gotardo, MG, in 1982 (Yorinori, 1994). Latest estimates showed that the disease was present in 99 counties affecting more than two million hectares and causing losses estimated at U$ 53 million (Yorinori, 2000). SDS has become an important limiting factor for soybean production in Brazil, being regarded as the most difficult disease to control. Differences in reaction among cultivars have been noted in the field, but reliable sources of resistance are not available yet.

The first symptoms are usually observed at the beginning of flowering. Symptoms on expanding leaves are similar to those on soybean mosaic virus. On mature leaves, spots of interveinal chlorosis evolve to necrosis, while the adjacent tissues, specially those along the veins, remain green (Sinclair and Backman, 1989; Roy et al., 1997), resulting in a mottled ("carijó") leaf symptom. Necrosis progresses more rapidly from the top to the lower leaves of the plant. Under severe disease, leaflets fall ,leaving the petioles sticking out. Severe infection can also result in abortion of flowers and pods and in reduction of seed weight (Sinclair and Backman, 1989; Roy et al., 1997). The root rot symptom is most visible on the main root and begin with a reddish spot or blotch on the cortical tissue and expands to the entire root changing its color from purplish-red, dark reddish-brown to almost black. Severely infected plants when

*Approved for publication by the R&D Head of Embrapa Soybean, as manuscript 041/2000.

pulled up are easily removed and almost devoid of lateral roots. Under moist soil, roots of dead plants may show profuse sporulation of fungus, becoming covered by a layer of blue mass of conidia. The vascular system acquires a pale brown to dark-brown color that extends several centimeters above the soil level. The discoloration of the vascular system is associated with interveinal chlorosis and necrosis of the leaf (Embrapa, 1999). Infection by *F. solani* f. sp. *glycines* seems to be favored by nodule formation by *Bradrhyzobium japonicum*. Under field condition and on soybeans artificially inoculated with *B. japonicum* ,the first root lesion usually begins where nodules are concentrated on the main root at about 3-5cm below the soil line. *Rhizobium* nodules also acquire the same red color in the presence of infection, apparently colonized by *F. solani* . sp. *Glycines*.

Several soybean genotypes have been tested for reaction to SDS ,and varying levels of resistance have been reported (Rupe et al., 1991; Stephens et al., 1993a; Melgar and Roy, 1994; Nelson et al., 1997; Hartman et al., 1997; Huang and Hartman, 1998; Leão et al., 1998). Resistance to leaf symptom in cultivar Ripley was attributed to a dominant nuclear gene Rfs (Stephens et al., 1993a). Molecular markers used to identify alleles associated with SDS field resistance in cultivar Forrest suggest that two chromosome segments are responsible for 34% of the phenotypic variability (Hnetkovsky et al., 1996). Two loci which are responsible for 56% of the phenotypic variability in recombinants derived from the Forrest and Essex (susceptible genotype) cross were also detected by molecular markers (Chang et al., 1996). The severity of SDS symptoms was assessed in 12 soybean cultivars inoculated with *F. solani* culture filtrate. Mild leaf symptoms were observed in cultivars D83-4499, Ripley, TN4-86, Hamilton and Bass (Jin et al., 1996). After being inoculated in the greenhouse, PI 567374 followed by PI 520733 revealed a smaller area under the disease progress curve when compared to the other assessed genotypes. PI 567374 seedlings showed lower levels of disease severity after immersion in pathogen culture filtrate (Huang and Hartman, 1998).

SDS is presently considered the most important soybean disease in Brazil due to control difficulties (Yorinori, 2000). The most adequate agronomic practice for reducing SDS impact is the use of resistant cultivars (Hnetkovsky et al., 1996; Chang et al., 1996; Kilo et al., 1997; Yorinori, 2000).

In this study, reactions of soybean cultivars to SDS were evaluated using different criteria for assessing disease severity based on leaf symptoms. Several thousand soybean lines are tested annually for reactions to SDS by Embrapa Soja at Londrina, but the results have not been consistent. The objective of this study was to define a quick and reliable method for scoring SDS severity following inoculation by the toothpick method , to evaluate 3-4 thousand reactions of soybean breeding lines annually.

## MATERIAL AND METHODS

Two experiments were carried out in a greenhouse at Embrapa Soja, Londrina, PR, Brazil. They were sown on different dates to verify the repetitivity of the first results over time. The first experiment was carried out in June/2000 to verify whether disease severity based on leaf interveinal chlorosis would be more accurate since clear differences were noted among soybean cultivars. The second experiment , sown in September/2000, was carried out to determine which of the previous methods reported in literature (Rupe, 1989; Melgar et al., 1994; Yorinori, 1996; Hartman et al., 1997; Huang and Hartman, 1998) was more practical and accurate, since there seems to be no consensus among the authors. A randomized complete block design with four replications was used in both cases. Highly susceptible FT Estrela soybean cultivars, and moderately resistant cultivars such as MG/BR 46 (Conquista), BRS Milena, MT/BR 45 (Paiaguás), MT/BR 47 (Canário) (Yorinori and Nomura, 1994; Costa and Yorinori, 1995), PI 5207333, PI 567374 (Huang and Hartman, 1998) and Ripley (Stephens et al., 1993a; Jim et al., 1996) were inoculated. Both inoculated with non-colonized toothpicks and inoculated FT Estrela cultivars respectively were used as controls. Plants were inoculated by the toothpick method (Yorinori, 1996) and assessed 21 days after inoculation. Twenty seeds were sown in each plastic pot containing 3kg of a greenhouse-mix soil (15% sand + 30% cow manure + field clay soil), thinned to 15 plants per pot at the VE stage (emergence stage) (Fehr and Caviness, 1997) and kept under mist irrigation installed 1.50m above the pots and at temperatures ranging from 25ºC to 28ºC.

### Inoculations

The toothpick inoculation method had been used previously by Keeling (1982) and Yorinori (1996) to assess soybean genotype reaction to stem canker and by Costa and Yorinori (1995) to assess the genotype

reaction to SDS. It was also chosen by this study for providing a quick and easy evaluation of large number of soybean lines in a short period of time. The isolate SDS-05 of *F. solani* f. sp. *glycines* was grown on plates containing PDA medium. Five millimeter mycelial discs, from five to seven days-old cultures were transferred to plates containing cornmeal medium (50g cornmeal + 10g agar/l) in which toothpicks were inserted. The plates were incubated at 27ºC ±1°C for 15 to 20 days, until the toothpick ends were completely colonized by the pathogen. The toothpicks were then inserted into the center of the hypocotyl at approximately 1.5cm below the cotyledon node of 12 to 15 days old soybean plants. Inoculated plants were kept under high moisture conditions for 48 hours, with intermittent spray irrigation.

**Assessment criteria**

Two disease reading criteria were used in the first experiment. The first was based on the number of plants showing: a) no symptom; b) leaves showing interveinal chlorosis (CL); and, c) necrotic leaves (NE). The percentage of affected plants (%AP) was calculated using the %AP = [NE + CL*0.5)*100]/ total inoculated plants formula (Yorinori, 1996). The second criterion took into consideration different chlorosis levels such as : a) low (CLL); b) intermediate (CLI); and, c) high(CLH). In this case, the %AP = [(NE + CLL*0.2 +CLI*0.5 + CLH*0.8)*100]/total seedlings inoculated formula was used. The genotype reaction was distinguished into five categories: a) resistant, (R) = 0% to 25%AP; b) moderately resistant, (MR) = 26% to 50%AP; c) moderately susceptible, (MS) = 51% to 75%AP; d) susceptible, (S) = 76% to 90%AP; and, highly susceptible, (HS) = above 90%AP (Yorinori, 1996).

In the second experiment, different assessment criteria were adopted. The first criterion was based on the %AP = (NE + CL*0.5)*100/total inoculated plants formula (Yorinori, 1996). Two other scales with grades ranging from 0 to 5 were used to assess leaf symptoms: a) the first: 0 = no symptoms; 1 = 1% to 10% leaf area affected (laa); 2 = 11%-30% laa; 3 = 31% - 40% laa; 4 = 41% -90% laa; and, 5 = >90% laa (Rupe, 1989); and, b) the second: 0 = no symptoms; 1 = <10% chlorosis, without necrosis; 2 = 10%-30% chlorosis, <10% necrosis; 3 = 11%-30% necrosis; 4 = 31%-70% necrosis, moderate defoliation; 5 = 70% necrosis, severe defoliation (Melgar et al., 1994). A third scale with grades ranging from 1 to 5 was used: 1 = no symptoms; 2 = development of slight symptoms with mosaic (1% to 20% of the leaf area affected - laa); 3 = development of moderate symptoms, interveinal chlorosis and leaf necrosis (21% to 50% of laa); 4 = high development of symptoms with interveinal chlorosis and necrosis (51% to 80% of laa) and 5 = severe interveinal chlorosis and necrosis and/or dead plants (81% to 100% of laa) (Hartman et al., 1997; Huang and Hartman, 1998).

**Data analysis**

Analyses of variance and mean comparisons (Duncan test at 5% significance) were performed on the assessed parameters, disregarding the non-inoculated and non-colonized toothpicks controls. The experiments were also submitted to a combined analysis (Campos, 1984) to evaluate the effects of the inoculation dates (experiments 1 and 2) and the assessment criteria of the first experiment.

## RESULTS AND DISCUSSION

**Experiment 1**

First SDS foliar symptoms were evident between the 12$^{th}$ and 15$^{th}$ day after inoculation. On the 21$^{st}$ day after inoculation, typical leaf symptoms were observed, with interveinal chlorosis and necrosis on the most susceptible plants. The non-inoculated and non-colonized toothpick controls did not manifest any symptoms, while the inoculated susceptibility standard, FT Estrela manifested severe disease symptoms. Results were considered homogeneous since a significant effect of the assessment criteria was detected on the different reactions shown by the cultivars. No difference was found in the percentage of affected plants (%AP) within cultivars whenever disease severity was assessed either by the levels of leaf chlorosis or regardless of levels of chlorosis. Cultivar reactions are shown in Table 1. Among the cultivars regarded as moderately resistant, Ripley and MT/BR 47 (Canário) showed moderately susceptible (MS) reactions.

**Experiment 2**

The first SDS symptoms were also observed on the 12$^{th}$ to 15$^{th}$ day after inoculation. Disease readings were taken on the 21$^{st}$ day after inoculation and the controls and FT Estrela (susceptible standard) showed

no symptoms and high susceptibility to *F. solani* f. sp. *glycines* (%AP = 99.75%), respectively (Table 2). Differences among the three assessment criteria could not be statistically compared because of the different scales adopted. However, it was noted that the %AP criteria allowed a higher level of detail for screening purposes compared to the other scales. Table 2 shows the cultivar reactions according to the different assessment criteria.

**Combined analysis of the experiments**

The %AP data allowed a combined analysis of the experiments, as the largest residue mean square (133.93) was less than seven times greater than the smallest one (28.54) (Banzatto and Kronka, 1989). No significant differences between the two experiments were found (Table 3) and no significant differences in individual cultivar performance were detected between experiments as well. The differences observed were for cultivars MT/BR 47 (Canário) and Ripley that showed smaller %AP in experiment 2 and cultivar FT Estrela that showed greater susceptibility in this experiment (>%AP).

**Assessment criteria**

The categorizing of the chlorosis symptoms into three classes was not required by the %AP criterion. Results from this assessment criterion were more laborious to obtain but not different from those that considered only the number of plants with chlorotic and/or necrotic leaves, regardless of the severity of the symptoms. According to the %AP, cultivar reaction in most cases coincided with the distiction provided by the Duncan mean test at the 5% level of probability, attesting the coherence of the reaction classification used for soybean stem canker (Yorinori, 1996).

The scales of Rupe (1989) and of Melgar et al. (1994) used in the second experiment were not in accordance with the present study. Only two classes of genotypes were distinguished by the Rupe's scale, while three categories, that of the controls (score 0), that of the susceptible control (score 3.75) and that of the remaining cultivars with the same score (score 3), were observed using the scale by Melgar et al. (1994).

The scales by Hartman et al. (1997), Huang and Hartman (1998) and the %AP were more accurate in differentiating cultivar reactions. The notes scale categorized the cultivars into three classes, according to the Duncan mean test ($P<0.05$), using five score scales that simplify the distinction of the reactions. The %AP, however, allowed for a greater level of detail within similar reaction types. Rank differences were observed for Ripley and Conquista in the %AP and in the 1 to 5 score scale (Hartman et al., 1997; Huang and Hartman, 1998) assessments. The score

**Table 1.** Reaction of eight soybean genotypes to inoculation with *Fusarium solani* f. sp. *glycines* by the toothpick method in a greenhouse experiment. Embrapa Soja, Londrina, PR, Brazil. 2000.

| Cultivar | % Affected Plants[1/] | | Reaction[2/] |
|---|---|---|---|
| | Regardless of chlorosis levels | Based on chlorosis levels | |
| FT Estrela (not inoculated) | 0.00 | 0.00 | - |
| FT Estrela (inoculated with non-colonized toothpicks) | 0.00 | 0.00 | - |
| FT Estrela | 89.16aA[3/] | 88.66aA | S |
| Ripley | 66.63abA | 60.70bA | MS |
| MT/BR 47 (Canário) | 53.52bcA | 54.29bA | MS |
| BRS Milena | 44.56bcA | 40.12bA | MR |
| PI 520733 | 38.61bcA | 36.48bA | MR |
| MT/BR 45 (Paiaguás) | 36.68bcA | 33.73bA | MR |
| PI 567374 | 32.79cA | 33.02bA | MR |
| MG/BR 46 (Conquista) | 30.52cA | 27.80bA | MR |
| Mean | 44.82A | 43.48A | |
| C.V.% | 25.82 | 28.95 | |

[1/] Original data transformed to arc sin ($\sqrt{x/100}$) for statistical analysis. [2/] Resistant, (R) = 0% to 25%AP; moderately resistant, (MR) = 26% to 50%AP; moderately susceptible, (MS) = 51% to 75%AP; susceptible, (S) = 76% to 90%AP; and, highly susceptible (HS) = over 90%AP (Yorinori, 1996). [3/] Means followed by the same lower case in the column and upper case letter on the line did not differ by the Duncan mean test ($P<0.05$).

**Table 2.** Reaction of eight soybean genotypes to inoculation with *Fusarium solani* f. sp. *glycines* by the toothpick method and assessed according to different scales in a greenhouse experiment. Embrapa Soja, Londrina-PR, Brazil, 2000.

| Cultivar | Disease Scoring Scale | | | % Affected Plants[4/] | Reaction[6/] |
|---|---|---|---|---|---|
| | 0 to 5[1/] | 0 to 5[2/] | 1 to 5[3/] | | |
| FT Estrela (not inoculated) | 0.00 | 0.00 | 1.00 | 0.00 | - |
| FT Estrela (inoculated with non-colonized toothpicks) | 0.00 | 0.00 | 1.00 | 0.00 | - |
| FT Estrela | 4.00a[5] | 3.75a | 4.25a | 99.75a | HS |
| MG/BR 46 (Conquista) | 1.75b | 3.00b | 2.25c | 40.33b | MR |
| Ripley | 1.50b | 3.00b | 2.25c | 40.11b | MR |
| MT/BR 45 (Paiaguás) | 2.00b | 3.00b | 3.00b | 37.87b | MR |
| BRS Milena | 1.75b | 3.00b | 2.50bc | 35.46bc | MR |
| PI 567374 | 1.50b | 3.00b | 2.00c | 28.83bcd | MR |
| PI 520733 | 1.25b | 3.00b | 2.00c | 24.44cd | R |
| MT/BR 47 (Canário) | 1.50b | 3.00b | 2.25c | 18.89d | R |
| Mean | 1.91 | 3.09 | 2.56 | 41.02 | |
| C.V.% | 28.83 | 5.71 | 15.93 | 12.81 | |

[1/] 0: no symptoms; 1: 1% to 10% leaf area affected (laa); 2: 11% to 30% laa; 3: 31% to 40% laa; 4: 41% to 90% laa and 5: >90% laa (Rupe, 1989).[2/] 0: no symptoms; 1: <10% chlorosis, without necrosis; 2: 10% to 30% chlorosis, <10% necrosis; 3: 11% to 30% necrosis; 4: 31% to 70% necrosis, moderate defoliation; 5: >70% necrosis, severe defoliation (Melgar et al., 1994).[3/] 1: no symptoms; 2: development of light symptoms with mosaic (1% to 20% of the leaf are affected - laa); 3: development of moderate symptoms, interveinal chlorosis and leaf necrosis (21% to 50% laa); 4: symptoms highly developed with interveinal chlorosis and necrosis (51% to 80% laa); 5: severe interveinal chlorosis and necrosis and/or dead plants (81% to 100% laa) (Hartman et al., 1997; Huang and Hartman, 1998).[4/] Original data transformed to arc sin ($\sqrt{x/100}$) for statistical analysis.[5/] Mean followed by the same letter in the columns did not differ by the Duncan mean test ($P<0.05$).[6/] Resistant (R): 0% to 25%AP; moderately resistant, (MR): 26% to 50%AP; moderately susceptible, (MS): 51% to 75%AP; susceptible, (S): 76% to 90%AP; and highly susceptible, (HS): over 90%AP (Yorinori, 1996).

**Table 3.** Combined analysis of the greenhouse experiments carried out in two sowing periods to investigate soybean genotype reaction to inoculation with *Fusarium solani* f. sp. *glycines*. Embrapa Soja, Londrina-PR, Brazil, 2000.

| Cultivar | % Affected Plants[1/] | | | Reaction[2/] |
|---|---|---|---|---|
| | Experiment 1 | Experiment 2 | Combined analysis | |
| FT Estrela (not inoculated) | 0.00 | 0.00 | 0.00 | - |
| FT Estrela (inoculated with non-colonized toothpicks) | 0.00 | 0.00 | 0.00 | - |
| FT Estrela | 89.16aB[3/] | 99.75aA | 96.32a | HS |
| Ripley | 66.63abA | 40.11bB | 53.49b | MS |
| BRS Milena | 44.56bcA | 35.46bcA | 39.97bc | MR |
| MT/BR 45 (Paiaguás) | 36.68bcA | 37.87bA | 37.27bc | MR |
| MG/BR 46 (Conquista) | 30.52cA | 40.33bA | 35.34c | MR |
| MT/BR 47 (Canário) | 53.52abA | 18.89dB | 35.19c | MR |
| PI 520733 | 38.61bcA | 24.44cdA | 31.30c | MR |
| PI 567374 | 32.79cA | 28.83bcdA | 30.79c | MR |
| Mean | 44.82A | 41.03A | 42.92 | |
| C.V.% | 25.82 | 12.81 | 20.94 | |

[1/] Original data transformed to arc sin ($\sqrt{x/100}$) for statistical analysis.[2/] Resistant (R): 0% to 25%AP; moderately resistant, (MR): 26% to 50%AP; moderately susceptible, (MS): 51% to 75%AP; susceptible, (S): 76% to 90%AP; and, highly susceptible, (HS): over 90%AP (Yorinori, 1996).[3/] Means followed by the same lower case letter in the column and by the same upper case letter on the line did not differ by the Duncan mean test ($P<0.05$).

assessment requires well trained personnel, as small mistakes can affect the results.

Given these conditions, the %AP and the 1 to 5 score scale (Hartman et al., 1997; Huang and Hartman, 1998) assessments were the most reliable since they can be used according to the investigator's choice and precision level required.

**Genotype x environment interaction**

No differences were detected between the experiments (sowing date effects). However, cultivars MT/BR 47 (Canário), Ripley, and FT Estrela were affected by genotype x environment interaction. The first two cultivars were less susceptible to SDS while the latter was more susceptible in the second sowing time. Soybean cultivar response to SDS is a genetically complex and low heritability trait (Stephens et al., 1993b; Chang et al., 1996; Prabhu et al., 1997; Hnetkovsky et al., 1996), which may have led to the observed results, since sampling involved 60 randomized plants/genotype (four replications of 15 plants).

**Cultivar reaction to SDS**

The reproduction of the well known SDS leaf symptoms (Leão et al., 1998; Gasperi, 2000) in the experiments confirmed the efficiency of the toothpick method for *F. solani* f. sp. *glycines* inoculation in soybean. The non-inoculated and inoculated with non-colonized toothpicks controls did not reproduce SDS symptoms, while the inoculated susceptible control was severely affected (%AP = 96.32) ,showing the typical disease symptoms.

The cultivars that showed greatest resistance were PI 567374, PI 520733, MT/BR 47 (Canário) and MG/BR 46 (Conquista), followed by MT/BR 45 (Paiaguás) and BRS Milena. PI 567374 and PI 520733 were also the best treatments in a previous report (Huang and Hartman, 1998). On the other hand, the cultivar Ripley, previously considered as resistant (Hnetkovsky et al., 1996; Stephens et al., 1993a), behaved as moderately susceptible (%AP = 53.49) in this study. This can be partially explained by the genotype x environment interaction effects. FT Estrela was highly susceptible to SDS, a finding that corroborates with previous results (Leão et al., 1998), and it can be used as a susceptible control in studies that involve assessment of soybean cultivar/genotype reaction/variability.

No cultivar was completely resistant to SDS; however, the majority were moderately resistant, probably due to the fact that SDS is a complex trait with polygenic resistance, which reduces the possibility of detecting immune genotypes (Stephens et al., 1993b; Chang et al., 1996; Prabhu et al., 1997; Hnetkovsky et al., 1996). Some of the cultivars may be used as sources of resistance to SDS.

## CONCLUSIONS

The toothpick inoculation method was efficient to reproduce SDS leaf symptoms and may be used to inoculate *F. solani* f. sp *glycines* in soybean genotypes.

The %AP (Yorinori, 1996) and the 1 to 5 score scale (Hartman et al., 1997; Huang and Hartman, 1998) were most efficient to assess soybean reaction to SDS, based on leaf symptoms.

The cultivar FT Estrela was highly susceptible to *F. solani* and may be used as susceptibility control in studies comparing SDS soybean genotype reactions and as a susceptible parent in crosses for genetic studies.

The PI 567374, PI 520733 and MG/BR 46 (Conquista) genotypes were the most resistant to SDS, being recommended in crosses for breeding for resistance.

Further genetic analyses are needed to determine the genes involved in the resistance of the cultivars tested. Field tests are also necessary to evaluate the reaction of these cultivars under natural and severe disease outbreak.

## ACKNOWLEDGMENTS

The authors wish to thank the Conselho Nacional de Pesquisa – National Research Council (CNPq) for the scholarship granted to the first author and also to Dr. José Erivaldo Pereira, José Francisco Ferraz de Toledo, Léo Pires Ferreira e Romeu Afonso Souza Kiihl for their helpful suggestions.

## RESUMO

**Reação de cultivares de soja à síndrome da morte súbita e métodos para seleção de resistência.**

Na safra 1999/2000, a síndrome da morte súbita (SMS) da soja, causada por *Fusarium solani* f. sp. *glycines*, afetou mais de dois milhões de hectares de

soja, em 99 municípios brasileiros e os prejuízos foram estimados em U$53 milhões. Uma das principais formas de controle é a resistência genética das cultivares. Uma das dificuldades para selecionar cultivares resistentes é a falta de um critério de avaliação que permita distinguir, com segurança, os diferentes níveis de reação das cultivares de soja. Este trabalho teve como objetivos definir critérios de avaliação de cultivares de soja à SMS e encontrar fontes de resistência, com base nos sintomas foliares. Os experimentos foram realizados em casa-de-vegetação, em duas épocas, com oito genótipos e quatro repetições. A inoculação foi feita pelo método de palitos-de-dente e as avaliações ocorreram 21 dias após as inoculações, sendo utilizados cinco diferentes critérios de avaliação. Os resultados indicaram que para a determinação da percentagem de plantas afetadas (%PA), que indica diferenças nas reações à SMS, a avaliação de classes de clorose não diferiu significativamente da que considerou apenas a incidência de folhas com clorose e/ou necrose. As escalas de %PA e de notas de 1 a 5 foram as mais indicadas para a avaliação da reação de cultivares de soja à SMS através de sintomas foliares. A cultivar FT Estrela foi altamente suscetível (%PA=96,32%), mostrando ser um bom padrão de suscetibilidade para experimentos que envolvam a avaliação da reação de genótipos à SMS. Os genótipos PI 567734, PI 520733 e MG/BR 46 (Conquista) foram os mais resistentes à SMS, com %PA de 30,79%, 31,30% e 35,34%, respectivamente, indicando a possibilidade da sua utilização em cruzamentos que visem resistência a essa doença.

## REFERENCES

Banzatto, D. A. and Kronka, S. N. 1989. Experimentação agrícola. (Agricultural experimentation). FUNEP, Jaboticabal. 247p.

Campos, H. 1984. Estatística aplicada à experimentação com cana-de-açúcar (Statistics applied to sugarcane experimentation). FEALQ, São Paulo. 292p.

Chang, S. J. C.; Doubler, T. W.; Kilo, V.; Suttner, R.; Klein, J.; Schmidt, M. E.; Gibson, P. T. and Lightfoot, D. A. 1996. Two additional loci underlying durable field resistance to soybean sudden death syndrome (SDS). Crop Science. 36(6):1684-1688.

Costa, C. L. and Yorinori, J. T. 1995. Métodos de inoculação de *Fusarium solani* para avaliação de resistência em soja. Fitopatologia Brasileira (Inoculation methods of *Fusarium solani* for screening for resistance in soybeans). 20:332.

Embrapa Soja. 1999. Recomendações técnicas para a cultura da soja no Paraná (Technical recommendations for soybean production in the State of Paraná 1999/2000) for 1999/2000. Londrina. 236p. (Embrapa Soja. Documentos, 131).

Fehr, W. R. and Caviness, C. E. 1997. Stages of soybean development. Iowa State University, Iowa. 12p.

Gasperi, A. C. 2000. Variabilidade de isolados, reação de cultivares e danos causados por *Fusarium solani* f. sp. *glycines* em soja (Variability of isolates, reaction of cultivars and damages caused by *Fusarium solani* f. sp. *glycines* on soybeans). M.S. Diss. Universidade de Passo Fundo, Passo Fundo. 93p.

Hartman, G. L.; Huang, Y. H.; Nelson, R. L. and Noel, G. R. 1997. Germoplasm evaluation of *Glycine max* for resistance to *Fusarium solani*, the causal organism of sudden death syndrome. Plant Disease. 81:515-518.

Hnetkovsky, N.; Chang, S. J. C.; Doubler, T. W.; Gibson, P. T. and Lightfoot, D. A. 1996. Genetic mapping of loci underlying field resistance to soybean sudden death syndrome (SDS). Crop Science. 36(2):393-400.

Huang, Y.H. and Hartman, G. L. 1998. Reaction of selected soybean genotypes to isolates of *Fusarium solani* f. sp. *glycines* and their culture filtrates. Plant Disease. 82(9):999-1002.

Jin, H.; Hartman, G. L.; Nickell, C. D. and Widholm, J. M. 1996. Phytotoxicity of culture filtrate from *Fusarium solani*, the causal agent of sudden death syndrome of soybean. Plant Disease. 80(8):922-927.

Keeling, B. L. 1982. A seedling test for resistance to soybean stem canker caused by *Diaporthe phaseolorum* var. *caulivora*. Phytopathology. 72:807-809.

Kilo, V. Y.; Abu-Thredeih, J.; Chang, S. J. C.; Gibson, P. T. and Lightfoot, D. A. 1997. Coinheritance of resistance to SCN and SDS in Pyramid x Douglas. Soybean Genetics Newsletter. 24:126-127.

Leão, R. Z. R.; Freitas, M. A. and Café Filho, A. C. 1998. Reação de cultivares de soja à inoculação com isolados de diferentes espécies e *formae specialis* de *Fusarium* (Reaction of soybean cultivars to inoculations with isolates of different species and

*formae specialis* of *Fusarium*). Summa Phytopathologica. 24(3/4):268-271.

Melgar, J. and Roy, K. W. 1994. Soybean sudden death syndrome: cultivar reactions to inoculation in a controlled environment and host range and virulence of causal agent. Plant Disease. 78(3):265-268.

Melgar, J.; Roy, K. W. and Abney, T.S. 1994. Sudden death syndrome of soybean: etiology, symptomatology, and effects of irrigation and *Heterodera glycines* on incidence and severity under fiel conditions. Canadian Journal of Botany. 72(11):1647-1653.

Nelson, B. D.; Hansen, J. M.; Windels, C. E. and Helms, T.C. 1997. Reactions of soybean cultivars to isolates of *Fusarium solani* from the red river valley. Plant Disease. 81(6):664-668.

Prabhu, R. R.; Doubler, T. W.; Chang, S. J. C. and Lightfoot, D. A. 1997. Development and utility of sequence characterized amplified regions (SCARS) from RAPD markers linked to SDS and SCN QTLs. Soybean Genetics Newsletter. 24:128-131.

Roy, K. W. 1997. *Fusarium solani* on soybean roots: nomenclature of the causal agent of sudden death syndrome and identity and relevance of *F. solani* form B. Plant Disease. 81(3):259-266.

Roy, K. W.; Rupe, J. C.; Hershman, D. E. and Abney, T. S. 1997. Sudden death syndrome of soybean. Plant Disease. 81(10):1100-1111.

Rupe, J. C. 1989. Frequency and pathogenicity of *Fusarium solani* recovered from soybeans with sudden death syndrome. Plant Disease. 73:581-584.

Rupe, J. C.; Gbur, E. E. and Marx, D. M. 1991. Cultivar responses to sudden death syndrome of soybean. Plant Disease. 75(1):47-50.

Sinclair, J.C. and Backman, P. A. (Eds.) 1989. Compendium of soybean diseases. 3.ed. APS Press, St. Paul. 106p.

Stephens, P. A.; Nickell, C. D. and Kolb, F. L. 1993. Genetic analysis of resistance to *Fusarium solani* in soybean. Crop Science. 33(5):929-930.

Stephens, P. A.; Nickell, C. D.; Moots, C. K. and Lim, S. M. 1993. Relationship between field and greenhouse reactions of soybean to *Fusarium solani*. Plant Disease. 77(2):163-166.

Yorinori, J.T. 1994. Podridão vermelha da raiz, uma séria doença da soja e do feijão, causada por *Fusarium solani)*. Fitopatologia Brasileira. 19:333.

Yorinori, J. T. 1996. Cancro da haste da soja: epidemiologia e controle (Soybean stem canker: epidemiology and control). Embrapa Soja, Londrina. 75p. (Embrapa Soja. Circular Técnica, 14).

Yorinori, J.T. 2000. Evolução da ocorrência e da severidade da podridão vermelha da raiz da soja (PVR/SDS) e reação das cultivares comerciais à doença. p.94. In: Resumos da XXII Reunião de Pesquisa de Soja da Região Central do Brasil, 22., Cuiabá, 2000.

Yorinori, J.T. and Nomura, S.L. 1994. Cultivares de soja resistentes à podridão vermelha da raiz causada por *Fusarium solani* [Soybean cultivars resistant to red root rot (SDS) caused by *Fusarium solani*]. Fitopatologia Brasileira. 19:339.

Received: September 03, 2001;

Accepted: March 20, 2002.