Field Resistance to Sudden Death Syndrome is effective at low Inoculum Concentration in Greenhouse Assays

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Abstract

Effective selection of field resistance to soybean (Glycine max (L.) merr.) sudden death syndrome (SDS) measured by leaf scorch requires multiple environments. Current greenhouse assays reduce GxE but fail to predict field resistance. Our objective was to develop a greenhouse assay with a low GxE that improved selection of field resistance to SDS among soybeans. Recombinant inbred lines were evaluated for scorch severity (DS) at three inoculum rates in two experiments and for root infection at one inoculum rate in one experiment. Cultivars were compared using DS from one experiment at one inoculum rate. The heritability of DS among recombinant inbred lines in the greenhouse was 63% at the low 35% at the moderate and 34% at the high inoculum rates. Reduced inoculum rates in the greenhouse (<5000 spores cm3 of soil) provided DS values that significantly correlated with field leaf scorch. The number of lines potentially resistant to SDS within a segregating population could be reduced by 57% using a F. solani inoculum rates of 3,500 - 5,000 spores per cm3 of soil and greenhouse DS <1.9 for selection. About 10% of field resistant lines were eliminated by error. Among unrelated soybean cultivars the low inoculum rate separated genotypes ($P < 0.05$) and their greenhouse DS significantly correlated with field DS ($r = 0.9$) and disease index ($r = 0.86$). Therefore the method is an effective tool for inheritance studies and cultivar trials.

Introduction

Soybean (Glycine max (L.) Merr.) sudden death syndrome (SDS) caused by Fusarium solani f. sp. glycines (Roy 1997) significantly reduces soybean yield in mid-western USA and South America (Wrather et al. 1997; Roy et al. 1997; Njiti et al. 1998a). Protection against yield loss derives from the use of SDS resistant cultivars (Gibson et al. 1994; Njiti et al. 1998a).

Selection for SDS resistance in the field is complicated by the quantitative nature of the trait and interactions between resistance loci and the environment (Njiti et al. 1996). Selection for stable and durable resistance to SDS might be improved using controlled environmental conditions in the greenhouse or growth chambers (Stephens et al. 1993a 1993b). However the existing greenhouse assays with seedlings have not been able to accurately predict field response of mature plants in inheritance studies (Torto et al. 1996).

The latent period of the disease differs between the field and greenhouse because the inoculum rate is higher (>10 000 spores/g of soil) in the greenhouse (Torto et al. 1996) than in the field (<3000 spores/g of soil; Roy et al. 1997). High pathogen concentrations overcome both partial and complete plant resistance to disease (Parlevliet 1979; Tooley and Grau 1982) including the soybean - F. solani interaction (Torto et al. 1996; Gray and Achenbach 1996; Hartman et al. 1997).

We compared selection for field resistance to SDS in the greenhouse by leaf scorch severity at three inoculum rates. The implications for breeding and biotechnology are considered.

Materials and Methods

The genetic material included 30 F5:10 recombinant inbred lines (RILs) (a subset of a population of 100 lines) from the cross of 'Essex' (Smith and Camper 1973) x 'Forrest' (Hartwig and Epps 1973) and 10 soybean cultivars of diverse genetic background. Essex is susceptible to SDS while Forrest is resistant to SDS (Gibson et al. 1994; Hnetkovsky et al. 1996). The RILs were selected by disease index (Gibson et al. 1994) mean to include three groups (10 RILs per group) that contrasted for SDS leaf scorch score by the mean of five field environments over five years (F5:6 to F5:10). The groups were: (1) field resistant; the 10 most resistant of the 100 lines eight of which were significantly more resistant than Forrest; (2) field partially resistant; the 10 of the 100 lines around the population median; and (3) field SDS-susceptible; the 10 least resistant of the 100 lines all of which were more susceptible than Essex; (Hnetkovsky et al. 1996). Although SDS resistance in the field is a partial resistance controlled by several genes "resistance" and "partial resistance" will be used to refer to groups 1 and 2 respectively in this paper.

The 10 diverse soybean cultivars included four that were susceptible to SDS in the field and six that were resistant to SDS in the field.

Four experiments were conducted in the greenhouse at the SIUC Horticulture Research Center in Carbondale IL. Two experiments evaluated the selected recombinant inbred lines for leaf scorch resistance by SDS disease severity (DS) at three inoculum rates (high=104 moderate=5x103 and low=3.3x103 spores per cm3 of soil). The third experiment evaluated recombinant inbred lines at a lower inoculum rate of 2x103 spores per cm3 of soil. One experiment evaluated diverse cultivars at 4x103spores per cm3 of soil. Experiments were conducted between November 1 1996 and March 1 1999. Plants were grown with a 14-hour photoperiod and the air temperature ranged from 20+2 oC at night and 27+2 oC during the day. Disease severity (DS) was rated at 21 days after inoculation on a scale of 1 to 9 (Njiti et al. 1996). Root infection severity was evaluated as colony forming units per gram of root.

Data were subjected to analysis of variance (ANOVA) (SAS Institute Inc. Cary NC). The lines within SDS group by experiment interaction was tested to justify pooling data from the first two experiments. Mean comparisons were made by LSD (Gomez and Gomez 1984). Correlations were determined using the CORR function of MSTATC (Freed et al. 1990).

Results and Discussion

Resistance to leaf scorch

SDS leaf symptoms were not observed on plants grown on non F. solani infested plant growth medium. There was a significant effect of inoculum rate in both experiments (Table 1). DS means were 2.8 for the low and 4.0 for the moderate inoculum rates in the first experiment and 2.1 4.0 and 7.2 for low moderate and high inoculum rates respectively in the second experiment. Higher F. solani inoculum rates were expected to cause more severe SDS leaf symptoms and greater root rotting (Gray and Achenbach 1996). Means of DS at the high inoculum rate in the first experiment could not be determined due to the death of numerous plants. Soil compaction contributed to the death of this set of plants (treatments were planted by different individuals) and was monitored closely in subsequent experiments.

Only at the low inoculum rate was there a significant difference ($P < 0.05$) among the three SDS groups of genotypes in SDS disease severity in both experiments (Table 2). The spore rate in the low inoculum treatment (3 500 spores per cm3 of soil) was similar to that in field hot spots (Roy et al. 1997). In the greenhouse the appearance of SDS symptoms occurred earlier (vegetative growth stages) than in the field (reproductive growth stages) due partly to adequate soil moisture and a restricted temperature range. Lines within groups of genotypes did not vary significantly in SDS disease severity in the greenhouse (Table 2). Since group members were selected by similarity of response to SDS in the field low variation within group was expected for an effective assay.

The significant difference between replications (Tables 1&2) in the second experiment and modest heritability (63%) indicated that the greenhouse assay did not control all environmental factors that can influence SDS occurrence and severity. Air temperature varied from bench to bench and could have influenced soil temperature which has been a major soil factor on SDS occurrence and severity in the field (Rupe et al. 1993). The use of growth chambers and water baths may enable us to test the effect of soil temperature on heritability.

There was no significant interaction between genotype group and experiment (Table 2). Therefore the two experiments were pooled for mean comparison. Within each genotype group SDS disease severity increased with increasing inoculum rate (Table 3). At the high inoculum rate all three groups were highly susceptible (Table 3) as expected (Stephens et al. 1993; Torto et al. 1996; Gray and Achenbach 1996). Therefore high inoculum rates may be responsible for the breakdown of field SDS resistance in the greenhouse (Torto et al. 1996; Stephens et al. 1993; Gray and Achenbach 1996 Hartman et al. 1997).

At the low and moderate inoculum rates the DS mean of the field SDS susceptible group was significantly higher than those of both the partially resistant and the resistant groups. However the field partially resistant and resistant groups were not significantly different from each other (Table 3). Although in the field the group of genotypes with partial resistance to SDS had a significantly higher DX mean (7.4%)than the group of genotypes with resistance to SDS (1.2%). Therefore the greenhouse assay was not as effective as a replicated field study for separating cultivars with partial

resistance from those with resistance.

The correlation between greenhouse disease severity and field disease severity was significant at the low ($r = 0.70$ $P = 0.0001$) and at the moderate ($r = 0.44$ $P = 0.02$) inoculum rates. There was also a significant ($P < 0.05$) correlation between greenhouse disease severity and field disease index at low inoculum rate. The strong correlation between field and greenhouse disease severities contrasted with the absence of correlations between field and greenhouse DS (Torto et al. 1996; Hartman Gibson and Lightfoot unpublished). Stephens et al. (1993) found a correlation between field and greenhouse DS among 12 cultivars that contrasted strongly for SDS field response but their greenhouse DS was significantly higher than field DS due to the higher inoculum rate in the root zone. Among the ExF recombinant inbred lines disease severity at the high inoculum rate did not correlate with any of the field disease parameters. Therefore inconsistencies between field and greenhouse responses may be caused by high inoculum rates in greenhouse assays.

The low inoculum rate treatment caused more variability among genotype groups as indicated by analysis of variance (Table 2) group means (Table 3) individual line means (Table 4) and correlation coefficients. The low inoculum rate provided SDS disease severity values only 35 to 60% greater than field values. The moderate inoculum rate was partly effective ($r = 0.44$ $P = 0.02$) in predicting field SDS response in the greenhouse. However mean DS was 2 to 3 times higher than in the field. Therefore inoculum rates of 3000 to 5000 spores per cm^3 of soil are recommended.

The DS mean for the resistant group (1.9) was used as the value denoting resistance in the greenhouse. While seven out of 10 resistant lines were predicted to be resistant by the greenhouse assay only one out of 10 susceptible lines appeared to be resistant. The partially resistant lines were predicted to divide equally between resistant and susceptible classes. Hence a 57% reduction in the number of soybean lines advanced can be achieved by greenhouse selection for resistance to SDS with about 10% elimination of resistant genotypes by error.

Resistance to root infection by Fusarium solani

In one experiment recombinant inbred lines transplanted onto soil infested

with 2,000 F. solani spores per gram in the greenhouse did not produce SDS leaf symptoms within 28 days suggesting the inoculum rate was too low to cause scorch. However F. solani root infection severity (IS) measured as colony forming units (CFU) per gram of dry root weight significantly correlated with disease severity from the low inoculum rate of the previous two experiments ($r = 0.37$ $P < 0.05$). The CFU means of SDS susceptible group (1,324) was significantly higher than on both the partially resistant (598) and the resistant (400) groups consistent with the disease severity at the low inoculum rate of two experiments that produced SDS leaf symptoms. Therefore IS may be effective in separating resistant from susceptible lines in the greenhouse when leaf scorch was absent and root resistance is segregating (Njiti et al. 1998b; Prabhu et al. 1999).

F. solani colonies were not recovered from roots of non-inoculated plants. Samples F. solani recovered from inoculated plants were used to reproduce SDS symptoms on greenhouse grown plants and so confirmed identification of the pathogen.

Robustness of the low inoculum rate assay

In a cultivar trial two out of four field susceptible genotypes were significantly more susceptible than all six field resistant genotypes. Three field SDS susceptible cultivars showed significantly ($P < 0.05$) higher SDS disease severity means than Ripley when evaluated in the greenhouse on soil infested with about 4000 F. solani spores per cm3 (Table 5). Among these ten cultivars greenhouse DS significantly correlated with field DS ($r = 0.9$) and field DX ($r = 0.86$). Given that most of these cultivars are not directly related to Essex and Forrest and because of the diverse germplasm they contain we conclude that the low F. solani inoculum rate greenhouse assay can select for field SDS resistance by leaf scorch in many genetic backgrounds.

The assay described above will allow for multiple cycles of testing per season and reduce the time to both produce and verify new resistant cultivars (Schmidt et al. 1999). The assay will reduce the costs of data loss due to field variability (Prabhu et al 1999). Finally the assay will facilitate the isolation of SDS resistance genes by characterization of recombinants during fine mapping (Meksem et al. 1999).

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