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RESEARCH

Charcoal Rot Disease Assessment of Soybean Genotypes Using a Colony-Forming Unit Index

Alemu Mengistu,* Jeffery D. Ray, James R. Smith, and Robert L. Paris

ABSTRACT

Charcoal rot [*Macrophomina phaseolina* (Tassi) Goid] of soybean [*Glycine max* (L.) Merr.] is a disease of economic significance throughout the world. Progress in developing resistant genotypes has been hampered because of a lack of reliable and efficient methods for assessment of soybean genotypes. Researchers need a common system for classifying soybean genotypes for their reaction to *M. phaseolina* that is consistent across environments. We propose a classification system based on a colony-forming unit index (CFUI), derived by dividing the colony-forming unit (CFU) value of a given genotype by the CFU value of a susceptible standard. Four other assessment methods were compared and correlated to CFUI: percent height of stem discoloration measured at R7, foliar symptoms taken at R7, area under the disease progress curve based on foliar symptom data collected four times during the growing season up to R7, and the intensity of internal root and stem discoloration taken at R7 (root and stem severity [RSS]). Twenty-four soybean genotypes in Maturity Groups III through V were evaluated in 2002 and 2003 in naturally and artificially infested fields. Based on the CFUI, four genotypes were classified as moderately resistant to *M. phaseolina*. Among the disease assessment methods, RSS had the highest correlation with CFUI (r = 0.71 in 2002 and r = 0.69 in 2003). The CFUI provided a good measure of disease resistance across environments but was still time consuming. The RSS provided a less-accurate but more-rapid alternative that may be suitable for some breeding strategies.

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Abbreviations: AUDPC, area under the disease progress curve; CFU, colony-forming unit; CFUI, colony-forming unit index; FS, foliar symptom rated at R7; PDA, potato dextrose agar; PHSD, percent height of stem discoloration; RSS, root and stem severity; SCN, soybean cyst nematode.

Charcoal rot [*Macrophomina phaseolina* (Tassi) Goid] of soybean [*Glycine max* (L.) Merr.] is a disease of economic significance (Smith and Wyllie, 1999). Charcoal rot occurs throughout the north central and southern regions of the United States as well as in tropical and subtropical regions of the world (Wyllie, 1988). When severe, the disease reduces both yield and seed quality (Smith and Wyllie, 1999). *Macrophomina phaseolina* is pathogenic to at least 500 plant species, including economically important crops such as corn (*Zea mays* L.), sorghum (*Sorghum* Moench), cotton (*Gossypium* L.), and tobacco (*Nicotiana*) (Su et al., 2001; Wyllie, 1988) in addition to soybean. Estimated soybean yield losses for 16 southern states averaged 8.54×10^5 tonne per year from 1974 to 1994, making charcoal rot the second most damaging disease in this region (Wrather, 1995).

Macrophomina phaseolina is considered a high-temperature pathogen, with severity of the disease increasing as air and soil temperatures increase (28–35°C), and when soil moisture becomes limiting (Gary et al., 1991; Pearson et al., 1984; Smith and Wyllie,

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1999; Wyllie, 1988). Aboveground symptoms of charcoal rot appear after flowering, particularly at the R5, R6, and R7 (Fehr et al., 1971) growth stages (Short et al., 1978; Wyllie, 1988). Diseased plants may wilt and prematurely die, with dead leaves remaining attached to petioles and petioles remaining attached to stems. Drought conditions favor the development of charcoal rot in sorghum and sunflower (Gary et al., 1991, Manici et al., 1995).

Efforts to manage charcoal rot in soybean through adjusting planting dates, crop rotation, planting densities, and irrigation have all been suggested as means of control (Bowen and Schapaugh, 1989; Francl et al., 1988; Ghaffer et al., 1969; Mueller et al., 1985; Todd, 1993; Wyllie, 1988). Other control methods, including fungicide applications to seed and soil and biological control using hyperparasitism, have had limited effects on disease severity in other crops (Ghaffer et al., 1969; Siddiqui and Mahmood, 1993). Host resistance may be the only feasible method to manage this disease (Bowen and Schapaugh, 1989; Bristow and Wyllie, 1984; Smith and Carvil, 1997; Smith and Wyllie, 1999). Only a few soybean genotypes, including 'Delta and Pineland 3478', 'Hamilton', 'Jackson II', 'Davis', and 'Asgrow 3715,' have been identified as either moderately resistant or tolerant (Smith and Carvil, 1997; Smith and Wyllie, 1999). Soybean genotypes with high levels of resistance have not been identified.

Identification of resistant genotypes has been limited because of the lack of an efficient disease assessment method and the lack of a consistent classification scheme across experiments and years. Quantification of microsclerotia in stem and root tissue as colony-forming units (CFU) may be useful as a disease assessment method (Smith and Carvil, 1997). However, it is time consuming, expensive and data may vary among years and locations. These limitations of CFU make it difficult to consistently evaluate large numbers of soybean genotypes. Additionally, it is difficult to compare genotypic CFU values across environments and research programs, which can result in inconsistent conclusions regarding susceptibility or resistance.

The lack of both a consistent resistance classification scheme and a fast and reliable method of screening have slowed efforts at identifying sources of resistance to charcoal rot among soybean genotypes and incorporating that resistance into improved genotypes. The objectives of this research were (i) to develop and evaluate a new classification system for soybean genotypic reactions to *M. phaseolina* based on a colony-forming unit index (CFUI), (ii) to compare four other *M. phaseolina* disease assessment methods to CFUI, and (iii) to identify new sources of resistance using the CFUI system.

MATERIALS AND METHODS

Inoculum Increase

Japanese millet (*Echinochloa frumentaceae* L.) seed (400 mL by volume) were soaked for 18 to 20 h in 4 L of a solution containing distilled water and 40 g of table sugar, 0.5 g of yeast extract, and 0.25 g of tartaric acid per liter. The solution was decanted, and the millet seeds were divided equally into five autoclavable bags. A tube 5 cm in diameter and 10 cm long was inserted halfway into each bag and tied securely with the bag. This was to facilitate the placement of culture plugs into the millet. Cotton plugs were inserted into each tube, and the samples were autoclaved at 121°C for 30 min. The autoclaved millet was then divided equally among 12 bags. One-week-old culture plugs of *M. phaseolina,* grown on acidified potato dextrose agar (PDA), were used to inoculate the millet. Thirty 0.5-cm-diam. plugs were placed into each bag, and each opening was then reclosed with the cotton plug. The bags were incubated at 30°C for 3 wk, with periodic shaking to spread the inoculum within the bags. After 3 wk, the millet was completely colonized and darkened with microsclerotia. The millet was allowed to air dry and then stored sealed plastic containers at 4°C until use.

Experimental Plot and Inoculation Method

Field plots were established at the Delta Research and Extension Center at Stoneville, MS (lat. 33°26′ N). The experimental design was a randomized complete block with three and four replications in 2002 and 2003, respectively. Plots were planted on 3 May 2002 and 7 May 2003. Each plot was five rows wide (0.61 m spacing) and 6 m long. The experiments were conducted in different fields each year, but each field had the same soil type (Bosket soils, fine-loamy, mixed, active, thermic Mollic Hapludallfs) and were located close together. The field was tilled with a disk-harrow and/or a spring-tooth field cultivator. Before planting each year, the field was divided into six grids, and 10 soil cores were collected per grid. The cores were analyzed to establish initial *M. phaseolina* inoculum level and to determine the presence or absence of soybean cyst nematode (SCN) (*Heterodora glycines* Ichinohe) and other nematodes.

Twenty-four soybean genotypes were selected from the Southern Uniform Test entries based on their high yield and good agronomic performance in prior years (Paris and Bell, 1999, 2000, 2001). Three genotypes were maturity group III, 7 were maturity group IV, and 14 were maturity group V. The 24 genotypes were sown at a rate of 26.4 seeds m^{-1} of row at a 2.5 cm depth using a planter retrofitted with seed cone attachments and a hydraulic depth-control system. The hoppers were calibrated to apply charcoal rot-infected millet seed in the furrow at a rate of 1 g per 30.5 cm of row. Plots were maintained weed free with pre-emergent and postemergent applications of labeled herbicides. Plots were not irrigated. Data on rainfall, relative humidity, pan evaporation, solar radiation, and soil temperature and degree-day accumulation were obtained from the Stoneville weather station website (Delta Research and Extension Center, 2007). Hourly measurements of water potential over the growing season were made with a watermark soil moisture sensor, and gypsum moisture block using a Watchdog Weather Station (Spectrum Technologies, Inc., Plainfield, IL).

Disease Assessment

Five methods of disease assessment were used in this study. The first method, denoted as root and stem severity (RSS), was assessed from 10 randomly selected plants per plot at the R7 growth stage. The plants were gently uprooted and RSS scored by longitudinally splitting the stem and taproot of each

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plant and visually rating the intensity of discoloration as well as the microscelorotial load covering the vascular and cortical tissue. The ratings were on a scale of 1 to 5, where 1 = no discoloration and 5 = highly discolored (Fig. 1). The scale for RSS was divided into four classifications (Paris et al., 2006): resistant (values of 1), moderately resistant (values >1 and ≤2), moderately susceptible (values >2 and <3), and susceptible (values 3–5).

The second method was also based on microsclerotial stem discoloration at growth stage R7. The height from ground level of internal vascular discoloration was divided by the stem height × 100 to determine percent height of internal stem discoloration (PHSD) attributed to charcoal rot.

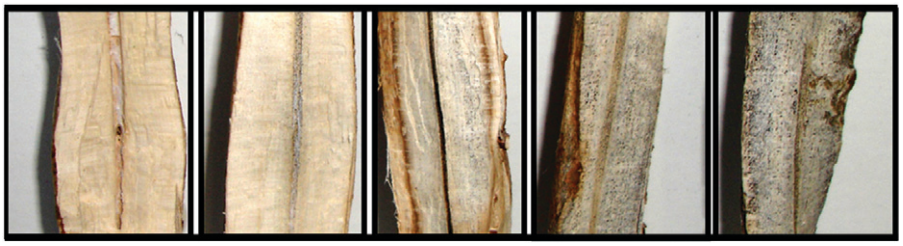

Figure 1. Split lower stem and root sections showing *Macrophomina phaseolina* microsclerotia evaluated for root and stem severity (RSS). On the scale 1 to 5, 1 = no microsclerotia visible in tissue; 2 = very few microsclerotia visible in the pith, vascular tissue or under the epidermis, vascular tissue has not discolored; 3 = vascular tissue is partly discolored and microsclerotia have partially covered the tissue; 4 = vascular tissue is discolored with numerous microsclerotia embedded in the tissue, microsclerotia are also visible under the outside epidermis in stem and root sections; and 5 = vascular tissue darkened due to high numbers of microsclerotia both inside and outside of the stem and root tissues.

The third method assessed foliar symptoms (FS) using the Horsefall-Barratt scale (James, 1974) at R7. The symptom that occurs at R7 is generally a necrosis of soybean leaves with the plants retaining their leaves rather than having normal abscission patterns. On the scale of 0 to 11 used for FS, 0 = no symptoms; 1 = 0 to 3%, 2 = 3 to 6%, 3 = 6 to 12%, 4 = 12 to 25%, 5 = 25 to 50%, 6 = 50 to 75%, 7 = 75 to 87%, 8 = 87 to 94%, 9 = 94 to 97%, 10 = 97 to 100%, and 11 = 100%. Using this scale, the genotypes were classified into four categories: resistant (zero), moderately resistant (>0 and <5), moderately susceptible (≥5 and <8), and susceptible (≥8).

The fourth disease assessment method was also based on foliar symptoms. Foliar symptoms were rated on a weekly basis beginning with the first onset of leaf symptoms up to the R7 growth stage. The early foliar symptoms of charcoal rot begin with necrosis and chlorosis of leaves in the upper canopy and then quickly progress to necrosis of the entire plant. The leaves remained attached to the plant even after the plant had died. A rating was given depending on the percentage of plants in each plot that were affected as well as the intensity of infection. The foliar symptoms over time were used to calculate area under the disease progress curve (AUDPC). We calculated AUDPC for foliar symptoms using the following formula (Tooley and Grau, 1984; Vanderplank, 1963):

$$\text{AUDPC} = \sum_{i=1}^{n-1}\left[\left(X_{i+1} + X_i\right)/2\right]\left(t_{i+1} - t_i\right)$$

where X_i = cumulative FS disease expressed as a proportion at the ith observation, t_i = time (days after planting) at the ith observation, and n = the number of observations of foliar symptoms.

The final method of disease assessment was based on CFUs. Samples that were uprooted for the RSS measurement were also used to determine CFUs. Samples for CFU were taken from the lower stem and root, including lateral and fibrous roots of each plant, by excising the stem and roots just below the cotyledonary node. Ten stem parts and roots from each plot were thoroughly washed and rinsed in water to remove soil, air dried, and stored at 25°C. The root and stem sections were ground with a UDY cyclone sample mill (Model 3010, UDY Corp., Fort Collins, CO) and passed through a 28-mesh screen (600-μm openings). The mill was thoroughly cleaned between samples with a suction device. For each sample, 0.005 g of ground tissue were placed in a Waring blender with 100 mL of 0.525% NaOCl for 3 min and collected over a 45-μm pore size sieve. The triturate was washed with sterile distilled water and then added to 100 mL of autoclaved PDA that had been cooled to 60°C and amended with rifampicin (100 mg L^{-1}) and tergitol (0.1 mL) (Pearson et al., 1984; Smith and Wyllie, 1999). After 3 d of incubation at 30°C, the CFU of *M. phaseolina* was counted and converted to CFU per gram of root and stem tissues. A CFUI was created by dividing the CFU for each genotype by the CFU for the genotype with the highest CFU (MD98-5579 in both years). The genotypes were then classified in percentage based on this CFUI as resistant (0 to <10), moderately resistant (10 to ≤30), moderately susceptible (>30 to 60), and susceptible (>60), in accordance with the classification system of Schmitt and Shannon (1992) developed for SCN.

Data Analysis

Analysis of variance was performed on data using SAS (SAS Institute, 1982). Data of the five disease measurements, CFUI, RSS, PHSD, FS, and AUDPC were analyzed using the general linear models statements, PROC REG, PROC MIX, and PROC CORR. Mean comparisons were made using Fisher's least significant differences.

RESULTS

Inoculum distribution of *M. phaseolina* was even throughout the field based on the samples taken before planting in which CFUs were not significantly different. The selected field sites had no history of SCN, but other plant parasitic nematodes were extracted from the soil with an average of 20 reniform (*Rotylenchulus reniformis*), two stunt (*Tylenchorhynchus* spp.), and two lesion (*Pratylenchus* spp.) nematodes per 200 cm^3 soil.

Soil temperatures measured at 5.1 cm depth were higher in 2002 than in 2003 for the months of June, July, and August (Table 1). Total precipitation for the months of June, July, and August was 189 and 287 mm in 2002 and 2003, respectively. The soil water potential data for June, July, and August was −140, −122, and −136 Kpa for 2002 and −125, −90, and −88 Kpa for 2003. The total pan evaporation for the months of June, July, and August in 2002 was 621 mm, whereas it was 468 mm in 2003. The accumulated degree days through August was 4070 and 4036 for 2002 and 2003, respectively.

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Table 1. Mean temperature, relative humidity, precipitation, soil water potential, soil temperature, pan evaporation, solar radiation, and accumulated growing degree days (GDD) for April through October 2002 and 2003 at Stoneville, MS.

Month	Mean max. air temp.	Mean max. relative humidity	Precipitation	Soil water potential†	Mean soil temperature at 5.1 cm depth	Pan evaporation	Mean solar radiation‡	Accumulated GDD T_{10}§
	°C	%	mm	Kpa	°C	mm	langleys d^{-1}	
2002								
May	28	97	72	−22	32	210	390	1187
June	32	99	63	−140	38	221	416	2060
July	34	100	63	−122	41	200	448	3082
August	34	100	63	−136	40	200	407	4070
2003								
May	29	87	65	−26	32	135	377	1227
June	31	91	130	−125	36	193	522	2038
July	33	93	63	−90	39	135	539	3021
August	35	94	63	−88	38	140	497	4036

†Data generated from a watermark soil moisture sensor, gypsum moisture block using a Watchdog Weather Station (Spectrum Technologies, Inc.). The rest of the weather data were generated from the Stoneville weather station (Delta Research and Extension Center, 2007).

‡A unit equal to one gram calorie per square centimeter of irradiated surface.

§GDD are calculated by taking the average of the daily maximum and minimum temperatures compared to a base temperature (10°C). As an equation, GDD = $T_{max} + T_{min} - T_{base}$.

Analysis of variance for CFUI indicated that there was no genotype-by-year interaction ($P < 0.05$). Colony-forming unit index values were therefore averaged across years for all genotypes. Values for CFUI ranged from 15 to 94% among genotypes (Fig. 2). None of the genotypes evaluated in this study were fully resistant to *M. phaseolina*, and all were colonized to some degree. However four genotypes were rated as moderately resistant, ten genotypes were rated as moderately susceptible, and ten genotypes were rated as susceptible.

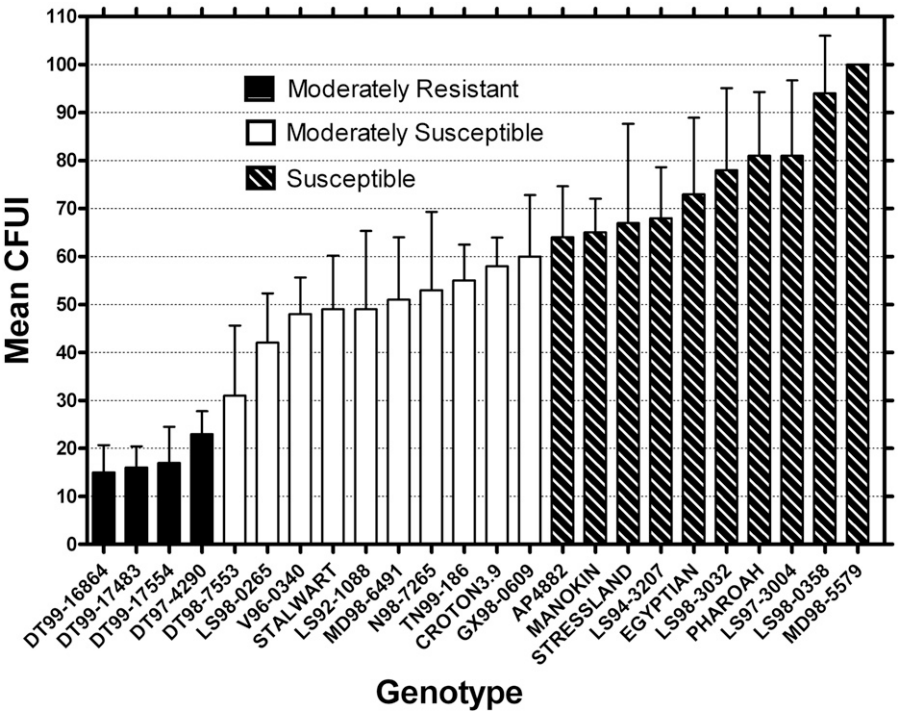

Figure 2. Colony-forming unit index (CFUI) of 24 soybean genotypes measured in 2002 and 2003. The CFUI was calculated by determining the ratio of the CFU values for each genotype to those of a common standard (MD98-5579). ANOVA indicated that there were no significant ($P > 0.05$) genotype-by-year interactions so the data for the 2 yr were averaged for each genotype. Genotypes are displayed from lowest CFUI value to the highest. Error bars represent the standard error of the mean. CFUI values less than 10 were resistant (none observed in this study), between 10 and 30 were moderately resistant (solid black bars), between 31 and 60 were moderately susceptible (solid white bars) and greater than 60 were susceptible (diagonally striped bars).

There was a significant ($P < 0.05$) genotype-by-year interaction for RSS, and RSS data for each year are therefore presented separately in Fig. 3. Based on RSS ratings in 2002, five genotypes were classified as moderately resistant, four genotypes as moderately susceptible, and the remaining 15 genotypes as susceptible. In 2003 8 genotypes were classified as moderately resistant, 11 genotypes as moderately susceptible, and 5 genotypes as susceptible. In neither 2002 nor 2003 were any genotypes classified as resistant. Root and stem severity values were moderately consistent ($r = 0.61$ and $P = 0.0015$) over years.

The PHSD method is a similar measure to RSS in that it measures internal stem discoloration caused by microsclerotia. However, it is based on the percentage of stem height discoloration rather than on discoloration intensity. Because there was a significant ($P < 0.05$) genotype-by-year interaction for PHSD, data by year are presented in Fig. 4. Percent height of stem discoloration ranged from 1 to 36% during 2002 and from 2.5 to 25% in 2003 (Fig. 4). At this time, no classification system has

been devised for PHSD. In 2002, however, five genotypes had PHSD values of <5%, another seven genotypes fell between 5 and 15%, and the remaining genotypes were all >15% PHSD. In 2003, five genotypes also had PHSD values of <5%. However, two of the five genotypes in 2003 were different from the five genotypes in 2002. All but five of the remaining genotypes fell between 5 and 15% PHSD in 2003. The PHSD values between the years were not well correlated ($r = 0.45$, $P = 0.0273$).

Analysis of variance indicated that there was a significant ($P < 0.05$) genotype-by-year interaction for FS. Therefore, data from each year is presented separately (Fig. 5). Four genotypes were classified as moderately resistant, 11 genotypes as moderately susceptible, and nine genotypes as susceptible in 2002. Ten genotypes were classified as moderately resistant, nine genotypes as moderately susceptible, and five genotypes as susceptible in 2003. Classification of individual genotypes varied between years. However, the correlation between years was moderate ($r = 0.53$, $P = 0.0071$).

Analysis of variance indicated a significant ($P \leq 0.05$) genotype-by-year interaction for AUDPC and data by year is therefore presented in Fig. 6. As with PHSD, no classification scale has been developed for AUDPC. However, there were 4 genotypes with AUDPC values <50, 10 with values between 50 and 100, and 9 with values >100 in 2002. There were eight genotypes with AUDPC values <50, three with values between 50 and 100, and the remaining 13 genotypes all had values >100 in 2003. Between the 2 yr, AUDPC values were not well correlated ($r = 0.45$, $P = 0.0290$).

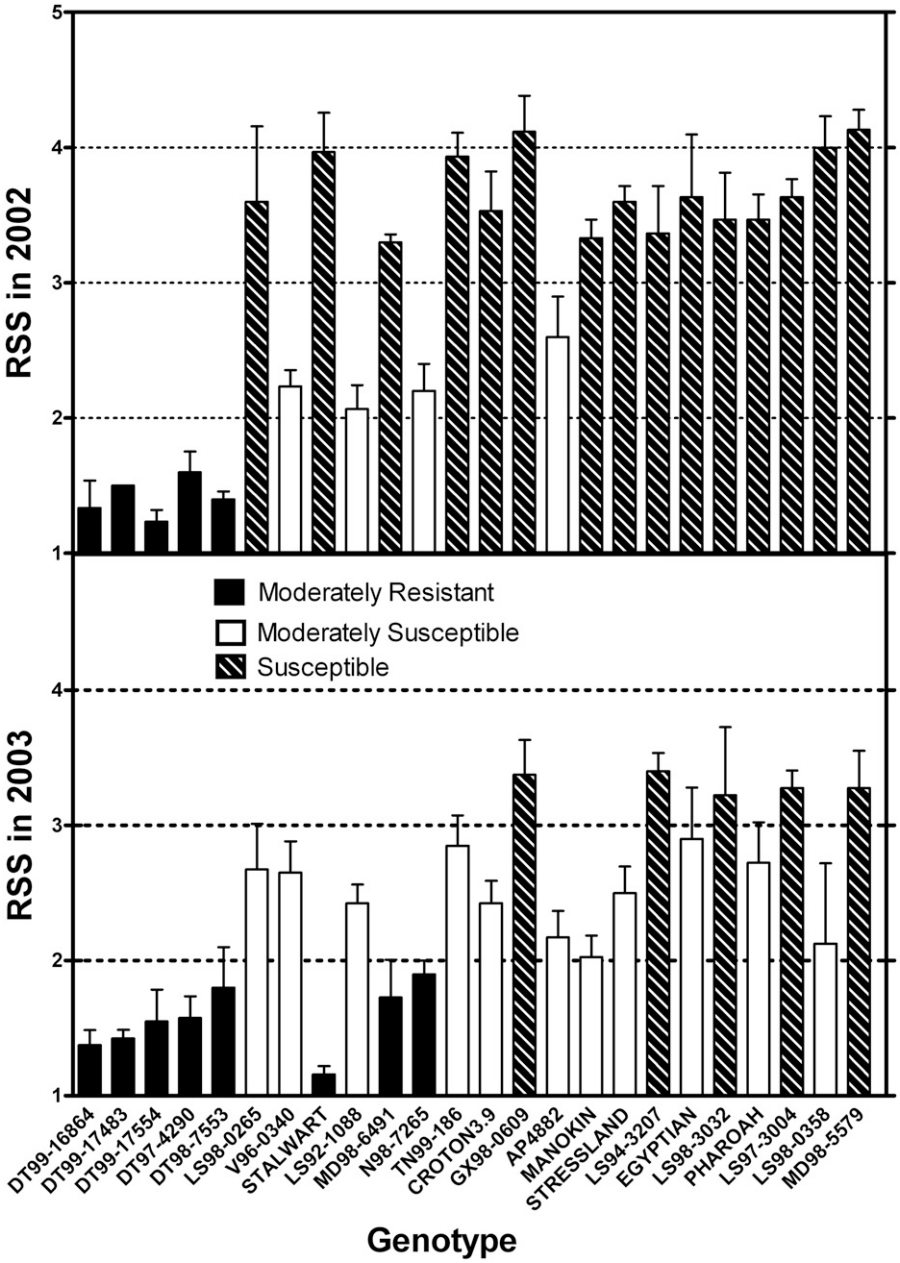

Figure 3. Measures of the intensity of internal discoloration (root and stem severity [RSS]) resulting from the presence of microsclerotia in the lower stem and root visually rated at R7. Data are arranged in the same order as Fig. 2. Error bars represent the standard error of the mean. Genotypes were classified on a rating of 1 to 5 based on the intensity of internal stem discoloration (Paris et al., 2006).

DISCUSSION

Except for CFUI, there was a significant year effect for each method. The other assessment methods indicated higher disease pressure in 2002 than in 2003. The higher overall levels of disease that developed during 2002 compared with 2003 could have been related to soil temperature. Soil temperatures measured at 5.1 cm depth were higher in 2002 (average 39.6°C) than in 2003 (average 37.6°C) for the months of June, July, and August. Soil temperature is an important component of the environment, and higher temperatures may increase disease severity. However, other environmental factors were also higher for these 3 mo in 2002, including air temperature, pan evaporation rates, and degree-day accumulation. Additionally, precipitation for the months of June, July, and August for 2002 was lower than in 2003. In short, the environmental conditions during 2002 were more favorable for causing higher disease scores than those during 2003.

Quantification of microsclerotia by CFUs in the lower stem and taproot tissues of soybean has been reported to be a reliable method of rating host compatibility between soybean genotypes and *M. phaseolina* (Smith and Carvil, 1997). One problem with such ratings however, is that CFU values can range from very low to tens of thousands, and the high values can vary greatly from year to year. Similar prob-

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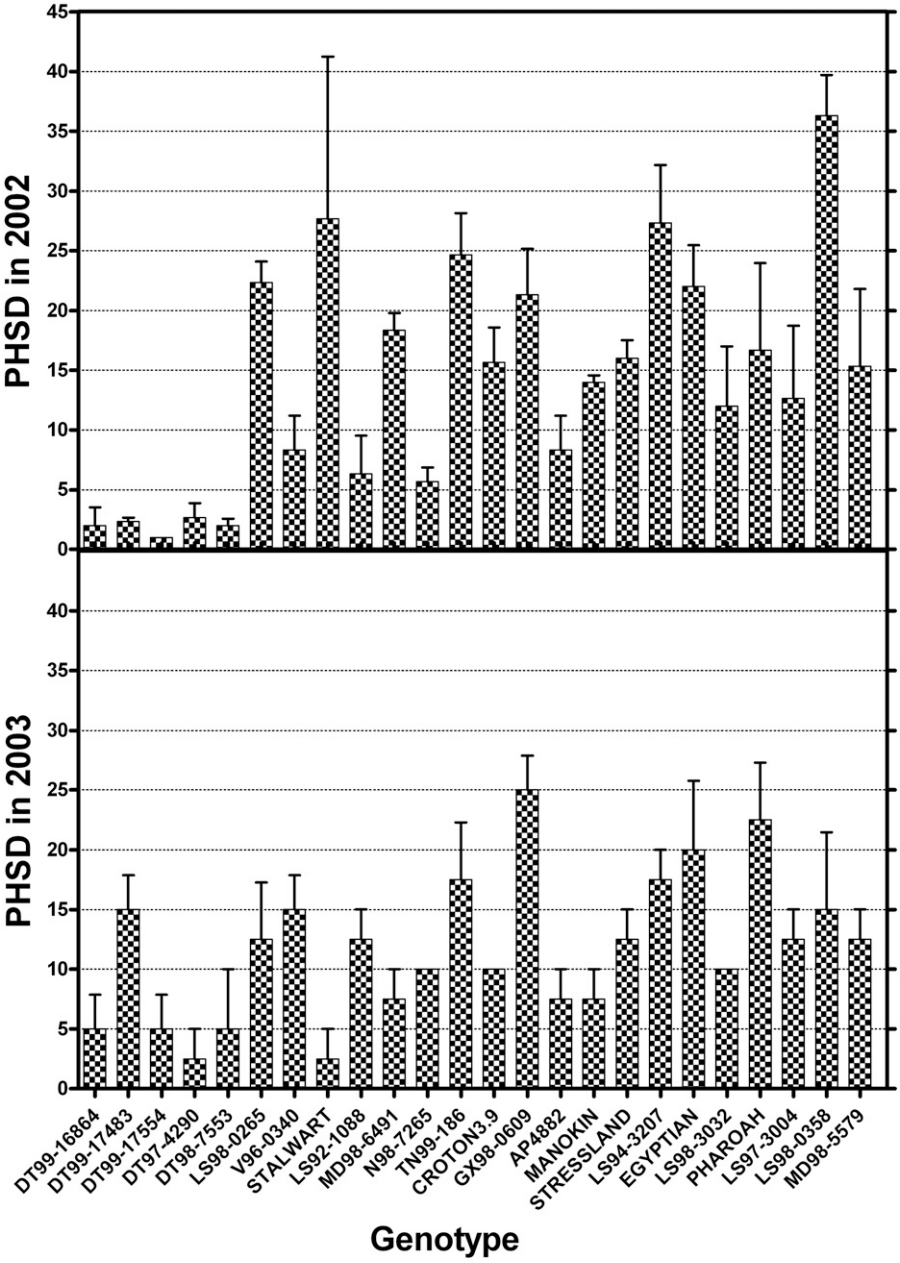

Figure 4. Measures of internal stem discoloration caused by microsclerotia based on the percent height of stem discoloration (PHSD) observed at R7. Data are arranged in the same order as Fig. 2. Error bars represent the standard error of the mean. Values are the percentage of total stem height that was internally discolored by microsclerotia.

lems were faced by researchers rating soybean genotypes for resistance to SCN. Schmitt and Shannon (1992) developed a practical classification system for communicating resistance and susceptibility to SCN based on an index of parasitism (Triantaphyllou, 1975). The idea of the index was to divide the number of cysts on each genotype in question by the corresponding number of cysts on a common susceptible genotype (× 100) and then classify the results into one of four categories, resistant = 0 to 9, moderately resistant = 10 to 30, moderately susceptible = 31 to 60, and susceptible >60. This system has been widely used within the soybean SCN research community and has been adopted in other crops, such as *Phaseolus vulgaris* (Smith and Young, 2003). In our experiment, we applied a similar procedure and scale to the analysis of the quantification of microsclerotia.

Adopting the system that Schmitt and Shannon (1992) developed for classifying the reaction of soybean genotypes to SCN to classifying the reaction of soybean genotypes to *M. phaseolina* provided a uniform and objective method for comparing data across years and across experiments. Smith and Carvil (1997), for example, rated soybean genotypic responses to *M. phaseolina* in field experiments using a combination of CFU and seed yield. They concluded that genotypes with low CFU values and high seed yield were more resistant to *M. phaseolina* than those with high CFU and low seed yield. We used the CFU data presented in Table 5 of Smith and Carvil (1997) and calculated a CFUI for each of their genotypes (Fig. 7) in the same manner as was done for the genotypes from our study shown in Fig. 2. The genotype they reported as having the highest CFU values (NK S31-33) was used as the common susceptible standard. The results indicated that no genotype was resistant, as was the case according to Smith and Carvil (1997). The same four genotypes classified as moderately resistant by Smith and Carvil (1997) were rated as moderately resistant with CFUI. However, the CFUI identified five genotypes as moderately susceptible, whereas Smith and Carvil (1997) had no intermediate classification.

The close agreement between the results reported by Smith and Carvil (1997) based on CFU and seed yield and our analysis of their data supports the use of the CFUI. A major advantage of using the CFUI over absolute CFU values is that CFU quantities can vary greatly over years and locations. A genotype with a low CFU value may actually be susceptible but classified as resistant if there is not an objective standard of susceptibility for comparison across assay environments. Hence, use of the CFUI is preferred because it provides objective criteria for rating soybean genotypes across years, locations, and experiments.

A critical aspect of each disease assessment method was how well it correlated with the CFUI (Table 2). The highest correlation with the CFUI occurred for AUDPC in 2003 (r = 0.74). However, the correlation for AUDPC in 2002 was low (r = 0.32). The correlation between the CFUI and PHSD was also different between years, being

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higher in 2002 (r = 0.63) than in 2003 (r = 0.44). Both AUDPC and PHSD had inconsistent correlations with the CFUI across years, as well as significant genotype-by-year interactions. The variation between years indicates a sensitivity to environmental conditions. In addition, since the years had opposite effects on the correlations of each parameter with the CFUI, it may be that PHSD and AUDPC are influenced by different environmental components. Hence, inconsistent correlations with the CFUI and the considerable variation between years for both PHSD and AUDPC limit their usefulness as reliable selection alternatives to using CFU.

The correlations between FS and CFUI were more consistent between years (r = 0.53 and 0.72 in 2002 and 2003, respectively) than were those of PHSD and AUDPC with the CFUI. Visual comparison of the results for FS (Fig. 5) and the CFUI (Fig. 2) shows that the ratings do not match very well in the critical area of determining moderately resistant genotypes. No resistant genotypes were identified by any methodology. In 2002 only one of the four genotypes identified by the CFUI as moderately resistant was classified by FS as moderately resistant. In a plant breeding program, this would have resulted in the elimination of 75% of the moderately resistant genotypes, with 75% of the remaining genotypes being moderately susceptible. This would represent poor progress indeed. In 2003, based on FS, 10 genotypes were classified as moderately resistant, including all four of the moderately resistant genotypes identified by CFUI. However, 60% of those classified as moderately resistant would have actually been moderately susceptible or susceptible according to the CFUI. Therefore, FS is not a reliable substitute for measuring CFU.

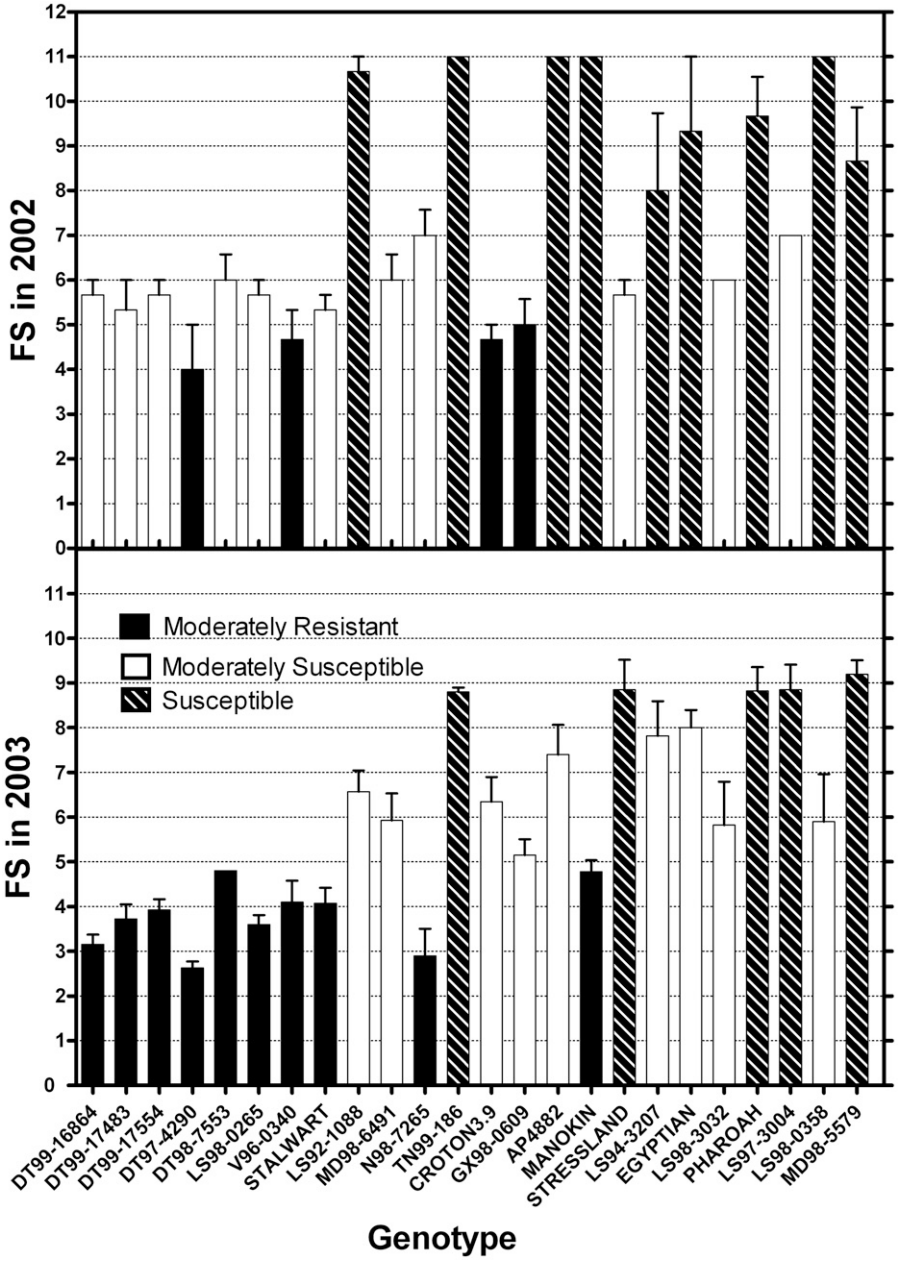

Figure 5. Measures of foliar symptoms (FS) visually rated at R7. Data are arranged in the same order as Fig. 2. Error bars represent the standard error of the mean. Genotypes were classified on a scale of 0 to 11.0, with values of 0 being resistant (none observed in this study).

The correlation between RSS and the CFUI was the most consistent across years (r = 0.71 and 0.69 in 2002 and 2003, respectively) and was moderately high in both years. The consistent and moderately high correlation between RSS and the CFUI may be because both methods are based on rating the intensity of actual fruiting bodies (microsclerotia) as the measure of disease severity. Visual comparison of the CFUI and RSS showed good agreement among moderately resistant classifications, especially in 2002. In 2002 one misclassified genotype (DT98-7553) would have been retained, but none of the moderately resistant genotypes (as determined by the CFUI) would have been eliminated. Again, in 2003 all moderately resistant genotypes would have been retained. However, four genotypes misclassified by RSS as moderately resistant would also have been retained. It is apparent that when using RSS in a less disease conducive environment, the distinction between resistance and susceptibility is not always clear. The data for the 2 yr indicate that RSS did a better job of correctly classifying moderately resistant genotypes in years when the environmental conditions favored severe disease development (as in 2002) than in less-severe years (as in 2003). This indicates that if RSS were to be used as a substitute for measuring CFU, replications, multiple years, multiple

generations, and/or multiple locations should be used. This would lower the likelihood of misclassification and allow for the elimination of previously misclassified genotypes. When more precise and accurate classification is needed, however, such as for genetic studies, RSS may not be adequate. Rather, the CFUI would be the recommended method.

CONCLUSIONS

The data presented in this paper indicate that the CFUI provides a consistent and objective method for classifying soybean responses to *M. phaseolina* across years and environments. Therefore, we propose that the indexing system described by Schmitt and Shannon (1992) for rating soybean response to SCN be adopted for rating soybean response to *M. phaseolina* using CFU to calculate the indices. Based on CFUI data, 4 genotypes (DT99-16864, DT99-17483, DT99-17554, and DT 97-4290), of the 24 evaluated, were rated as moderately resistant to *M. phaseolina*.

Our data further indicate that successful field screening of soybean genotypes for resistance to *M. phaseolina* may be achieved using the RSS method of disease assessment. The RSS method may have utility in breeding programs where there is a need to evaluate large numbers of breeding lines for resistance against *M. phaseolina*.

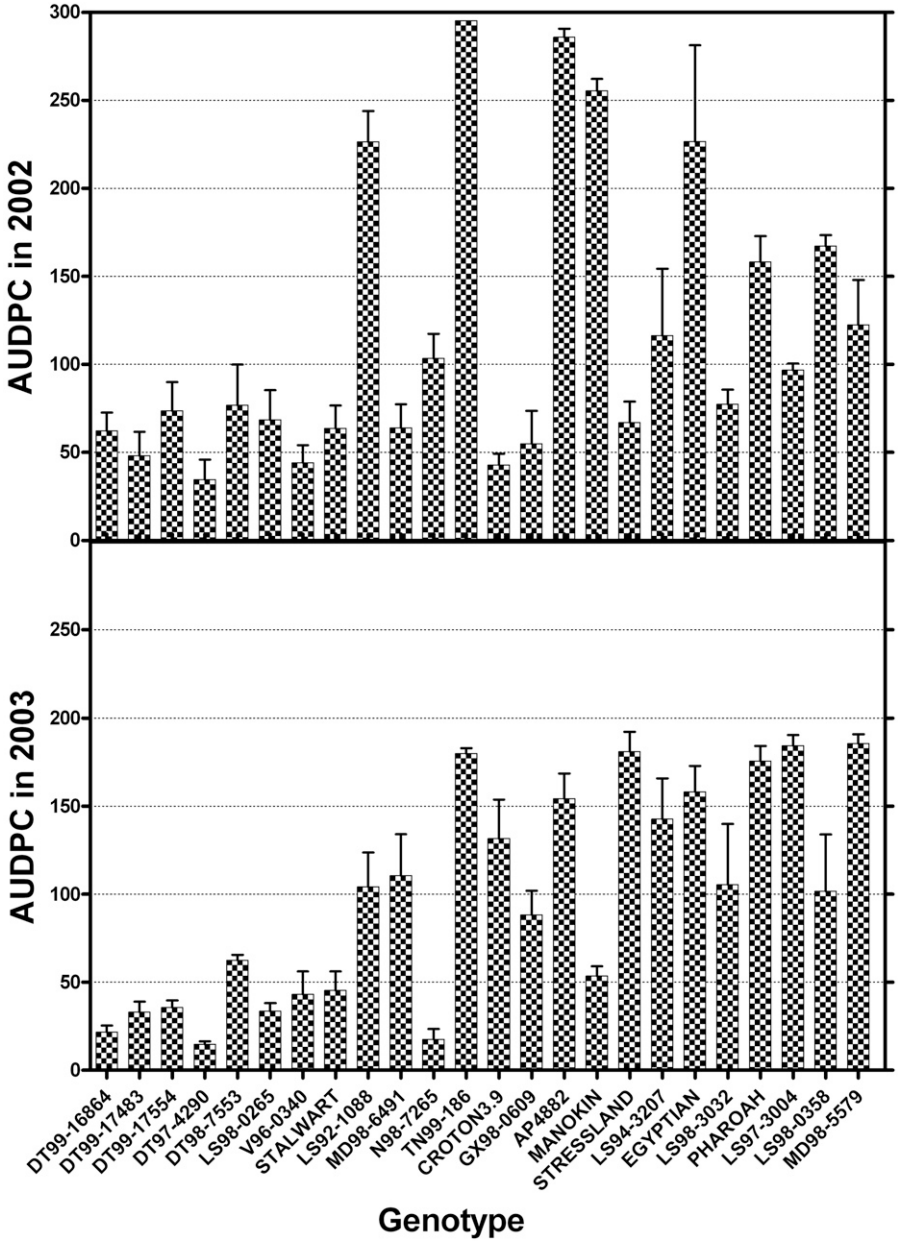

Figure 6. Weekly scoring of foliar symptoms from the onset of visual symptoms to R7, reported as the area under the disease progress curve (AUDPC). Data are arranged in the same order as Fig. 2. Error bars represent the standard error of the mean.

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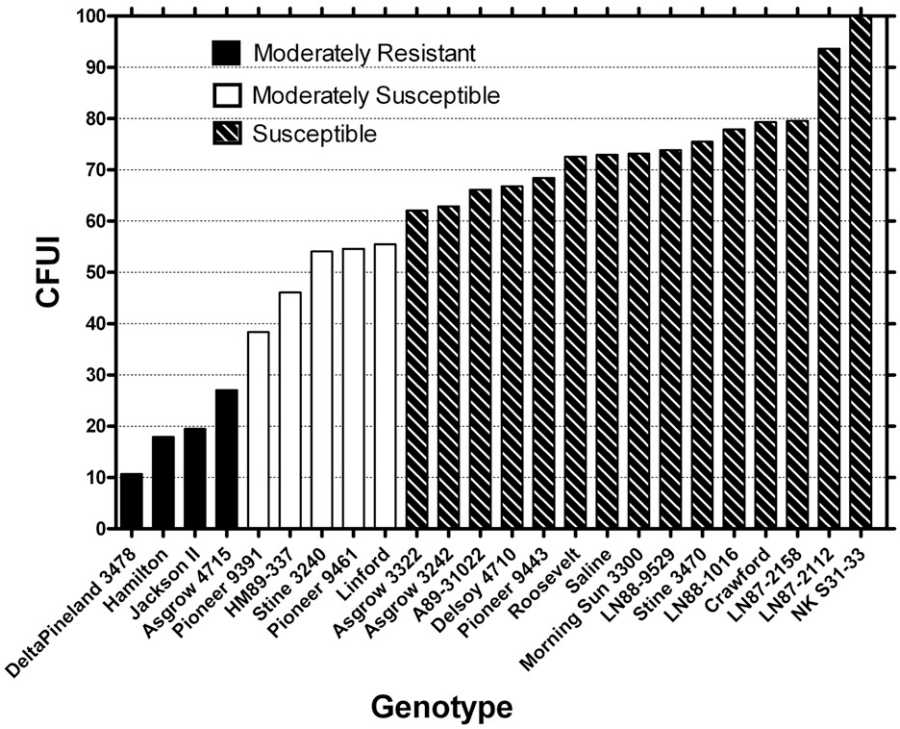

Figure 7. Colony-forming unit (CFU) data from Table 5 of Smith and Carvil (1997) converted to colony-forming unit index (CFUI) using CFU values for genotype NK S31-33 as the common standard. Data are arranged from lowest CFUI value to the highest. The classification scale employed for CFU Index was the same as in Fig. 2.

Table 2. Correlation matrix of five *M. phaseolina* disease assessment methods evaluated in 2002 and 2003.

Parameter	CFUI†	RSS†	FS†	PHSD†	AUDPC†
			2002		
CFUI	1.00	0.71***	0.53**	0.63***	0.32 NS‡
RSS		1.00	0.30 NS	0.86***	0.23 NS
FS			1.00	0.33 NS	0.94***
PHSD				1.00	0.24 NS
AUDPC					1.00
			2003		
CFUI	1.00	0.69***	0.72***	0.44 *	0.74***
RSS		1.00	0.67***	0.71***	0.67***
FS			1.00	0.50*	0.99***
PHSD				1.00	0.49*
AUDPC					1.00

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

†CFUI, colony-forming unit index, is the ratio of the colony-forming unit values for each genotype to those of a common standard (MD98-5579); RSS, root and stem severity, is based on a 1–5 scale, where 1 = no microsclerotia visible in tissue and 5 = vascular tissue darkened due to high numbers of microsclerotia; FS, foliar symptom rated at R7; PHSD, percent height of stem discoloration recorded at R7; AUDPC, area under the disease progress curve, is a weekly scoring of foliar symptoms from the onset of visual symptoms to R7.

‡NS, nonsignificant.

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