Field evaluation and genetic variation of some novel soybean cyst nematode resistance sources

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Introduction

The soybean cultivars currently used in the US have very low genetic diversity because they derived from a limited number of plant introductions (PI) (Lorrenzen and Shoemaker, 1994; Shoemaker, 1994). Soybean PIs come from Asia, and have historically been used either directly as varieties after evaluation or as sources of desirable genes for soybean breeding programs (Caviness, 1992). They have also been used in the US as sources of resistance to important diseases including Soybean Cyst Nematode (SCN), caused by *Heterodera glycines* Ichinohe, and Soybean Sudden Death Syndrome (SDS), caused by *Fusarium solani* (Mart.) Sacc. f. sp. *phaseoli* (Burk.) Snyd. & Hans., type A (Fehr, 1993; Palmer et al., 1996; Rao-Arelli, 1994; Rao-Arelli et al., 1992). These two infectious diseases often occur jointly in the field, resulting in important yield losses.

Generally, the PIs used in soybean breeding programs as sources of resistance against SCN have a black seed coat, poor agronomic performance,

and are also very susceptible to SDS in the field (Gibson et al., 1994; Gelin, 1996). Breeders are in search for new sources of SCN resistance in order to expand the genetic diversity of SCN resistance and at the same time to develop cultivars with combined resistance to both diseases.

In this research, 31 new SCN-3 resistant plant introductions (P. R. Arelli, personal communication) were evaluated at two different locations in Southern Illinois in 1995, along with 11 previously used SCN resistance sources and 40 cultivars used as controls. The objectives of the study were: (i) to evaluate the agronomic performance of the new PI lines for flower color, flowering date, harvest maturity, yield and seed quality; (ii) to estimate their SDS response in the field; and (iii) to assess their genetic variation with the use of two simple sequence repeats (SSR) DNA markers.

Materials and Methods

Field Evaluation

Fields with high productivity and a history of severe SDS infestation were chosen in Ridgway, Gallatin county, and Ullin, Pulaski county in southern Illinois. A rectangular lattice design was used at both locations, with two replications. The plots were randomized using MSTAT-C (Freed et al., 1989). Along with the 31 new PIs (PI567. series), 11 old PIs and 40 known cultivars were included in the trials as checks, giving 82 plots per replication. The plots were 0.76 cm apart, and had two rows of 4 m each, which were later end-trimmed to 3 m. The fields

were planted on 24 May, 1995 in Ridgway, and on 5 June, 1995 in Ullin. Harvest date was dependent upon the maturity group of the lines.

Agronomic performance

Flower color was recorded at flowering date, and flowering date was recorded as the first day when flowers were visible on 50% of the plants in a plot. Maturity (number of days from planting until suitable to combine) was scored at or close to harvest time. After harvest, the seed was cleaned and seed yield was measured in kg per plot at the Agronomy Research center (ARC-SIUC), and later converted into t ha^{-1}. Seed quality was estimated from a 30 g sample collected from each plot, and the following characteristics were recorded: seed number and color, hilum color and hilum color extension over seedcoat (0 = none to 5 = considerable extension).

SDS Data collection

Disease incidence (DI) was recorded per plot as the percentage of plants showing visible leaf symptoms. Disease severity (DS) was also rated from symptomatic plants only, using a scale of 1 to 9, where 1 = 0 - 10% total of leaf area necrotic, 2 = 10 - 20% chlorotic or any necrosis up to 10%, 3 = 20 - 40% chlorotic or 10-20% necrotic, 4 = 40 - 60% chlorotic or 20-40% necrotic, 5 = Greater than 60% chlorotic or greater than 40% necrotic, 6 = Premature leaf drop up to 1/3 defoliation, 7 = Premature leaf drop from 1/3 to 2/3 defoliation,

8 = Premature leaf drop greater than 2/3 defoliation, and 9 = Premature death. The disease index (DX) was later estimated according to Gibson et al. (1994) using the formula DX = (DI*DS) / 9, with a range of 0 (no disease) to 10 (all plants dead before R6). The square root of the disease index (SQRTDX) was calculated and used in the statistical analysis.

Assessment of genetic diversity using microsatellite markers

Only 40 lines and cultivars out of the 82 used in the study were tested with DNA markers. These include the 31 new PIs, plus 9 old PI lines and checks used previously in breeding programs, which served as controls. Young leaves were collected at Ullin from the chosen lines, and kept at -70 °F before use. Total genomic DNA was isolated as previously described (Dellaporta et al., 1983). DNA concentration was measured with a fluorometer and diluted to 15 ng/µl for further use in PCR reactions. PCR amplification and fragment analysis were performed as previously described (Akkaya et al., 1995).

Because the new PI lines are resistant to soybean cyst nematode race-3, an evaluation of their genetic diversity would require the use of various types of phenotypic and molecular markers. In this study, however, only two microsatelite markers or simple sequence repeat (SSRs) were chosen to serve as a starting point for the molecular evaluation of these lines. SoyABAB with the core motif $(AT)_{20}$ and Sat_038 with the core motif $(AT)_{21}$ were used collectively in an attempt to estimate the genetic diversity of the new sources. SoyABAB has previously been mapped to the linkage group 24, whereas Sat_038, previously mapped to

the linkage group 7, is known to be tightly linked (approximately 2.4 cM) to the major SCN resistance gene *rhgI* identified in soybean accessions (Akkaya et al., 1995; Mudge et al., 1996; Yuan et al., 2002). The software NTSYSpc (Rohlf, 1997) was used to create a dendrogram for the lines using a similarity coefficient.

Statistical analysis

Linear interpolation was used to estimate DI and DS of each plot at R6.2 stage, and DX was then calculated. A two-way analysis of variance (Little and Hills, 1978) was performed using MSTAT-C (Freed et al., 1989) for the square root of the disease index (SQRTDX), flowering date, maturity and yield.

Results and Discussion

Agronomic performance of the new PI lines

Flower color, flowering date and maturity

Nineteen of the new PI lines were cataloged as MG IV, whereas MGs II, III and V had 4 sources each. Across all MGs, 16 lines had white flowers, and 15 had purple flowers. Apart from the MG II lines that have only white flowers, the two colors were mixed with different frequencies within the other MGs. Flowering date was strictly MG dependent, and generally more days were needed for complete flowering and harvest maturity (Figure 1). Among the 4 MG V lines, only 2 were scored, but they matured earlier than expected. Flower color, flowering date, and pubescence of the new PI lines are presented in Table 1.

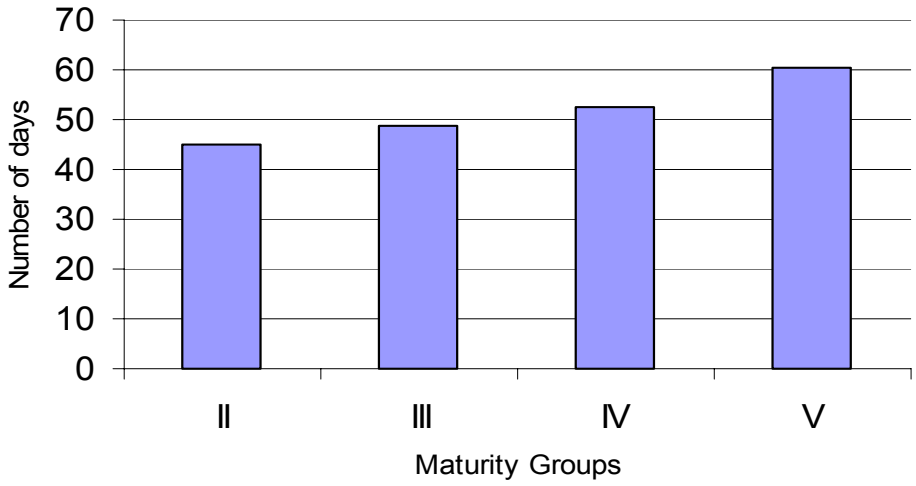

Figure 1. Comparison of flowering date of the new PI lines by maturity group (MG).

Yield of the new PI lines

The across location means of the four MG II PI lines were not statistically different (*P*>.05), and varied from 1.6 to 2.1 t ha^{-1}. These values were numerically lower than the yields of the checks 'Burlison' and 'Chapman', which had 2.7 and 2.8 t ha^{-1}, respectively. In the case of the four MG III lines, the yields were not statistically different (*P*>.05), and varied from 1.4 to 2.7 t ha^{-1}. The cultivar 'Linford' used as a check had the highest yield mean of 3.8 t ha^{-1}. Among the MG IV lines, significant differences were observed for yield (*P*<.05); the lowest mean was 0.3 and the highest was 2.6 t ha^{-1}. Some new lines had higher yield than several controls including 'Peking' which had an average yield of 1.1 t ha^{-1}, and 'Spencer' which had an average yield of 2.3 t ha^{-1}. The means of the four MG V lines were not significantly different and they varied from 0.9 to 1.6 t ha^{-1}.

Table 1. Flower color, flowering date*, and pubescence of the new PI lines

PI line	Flower color	Flowering date	Pubescence
	Maturity Group II		
PI567.418A	White	42	Gray
PI567.507B	White	48	Gray
PI567.364	White	48	Gray
PI567.512B	White	43	Gray
	Maturity Group III		
PI567.363B	White	47	Gray
PI567.491A	Purple	50	Tawny
PI567.365	White	51	Tawny
PI567.510A	Purple	48	Tawny
	Maturity Group IV		
PI567.336B	Purple	51	Tawny
PI567.568A	Purple	50	Tawny
PI567.581	White	53	Tawny
PI567.583D	White	51	Gray
PI567.636	Purple	57	Gray
PI567.286	Purple	48	Tawny
PI567.285	Purple	46	Tawny
PI567.421	Purple	55	Gray
PI567.577	White	54	Gray
PI567.303A	White	51	Tawny
PI567.415A	Purple	56	Tawny
PI567.492	White	51	Gray
PI567.336A	Purple	51	Tawny
PI567.562A	White	51	Gray
PI567.445B	Purple	51	Tawny
PI567.535A	White	53	Tawny
PI567.373A	White	51	Gray
PI567.516C	Purple	52	Tawny
PI567.583C	White	48	Tawny
	Maturity Group V		
PI567.373B	Purple	62	Tawny
PI567.342	Purple	56	Tawny
PI567.568B	White	64	Gray
PI567.660B	White	60	Gray

* Flowering date was the first day when 50% of the plants in a plot had flowers.

Seed number and color

The average number of seeds recorded varied from 121 to over 700 seeds per sample of 30 g drawn from each line. Seed number tended to increase in the later MGs. The explanation is that these lines also had smaller seed size, one trait that was apparently associated with black and green seed coat. With regard to seed color, more than 3/4 of the new lines had yellow seed coat (77.41%, 24 out of 31) with a brown hilum, the rest having either a black or a green seed coat. This relatively new association between yellow seed coat and SCN resistance is of great importance, considering the possibility for breeders to include some of these lines directly into specific breeding programs. In most old sources, SCN resistance was strongly associated with black seed coat. This undesirable linkage requires breeders to make numerous backcrosses in order to isolate those genotypes in which the linkage has been broken, so that the plants carrying only the SCN resistance genes and yellow seed coat could be identified and isolated for further investigation (Fehr, 1993). Because of their yellow seed coat, the new PI lines can be used in breeding programs as parents, mainly as SCN resistance sources, and in some instances as new SDS resistance sources as well.

Hilum color and hilum color extension

The hilum color of the new PI lines was generally brown, and only a few lines had a black hilum (Table 2). The extension of the hilum color over the seed

coat was generally moderate, except for a few lines in maturity group IV. In these lines, the hilum color was predominantly black.

Table 2. Seed characteristics of the new PI lines by maturity group

Maturity Group	Seed Color	Hilum Color	Hilum Color Extension*
II	Yellow	Brown, Black	0.5 – 3.0
III	Yellow, Black	Brown, Black	2.0 – 5.0
IV	Yellow, Black, Green	Brown, Black	0.0 – 5.0
V	Yellow, Green	Brown, Black	1.0 – 3.0

* 0 = none, 5 = considerable extension, rated on average of normal seeds.

SDS response of the new PI lines

The effect of SDS on the performance of the new PI lines was largely determined by the environment. At Ridgway, an overall mean of 4.2 was associated with a variance of 5.4, whereas the mean at Ullin was 2.0 and the associated variance was 1.4. The lines differed also in their response to SDS based on their respective MG. The earlier lines generally had a lower SQRTDX mean, which could be explained by a shorter life cycle in the field. Some lines had a very low SDS score, which is encouraging given that they are already resistant to SCN in the filed. A grouping of the new PI lines based on their SDS response is presented in Table 3.

Genetic diversity of the new PI lines

The microsatellite marker SoyABAB has separated 19 of the 40 lines into 3 different clusters, each containing both old and new sources. There was no amplification product for the remaining 21 lines and cultivars. Only one of these

Table 3. Classification of the new PI lines by their SDS response using SQRTDX

Number of lines	PI lines and their respective MG
	Class A: < 1.0
2	PI567.507B (MG II), PI567.418A (MG II)
	Class B: 1.0 – 2.0
6	PI567.512B(MG II), PI567.364(MG II), PI567.365(MG III), PI567.363B(MG III), PI567.445B(MG IV), and PI567.583C(MG IV)
	Class C: 2.0 – 3.0
5	PI567.491A(MG III), PI567.636(MG IV), PI567.415A(MG IV), PI567.373A(MG IV), and PI567.660B(MG V)
	Class D: 3.0 – 4.0
5	PI567.568A(MG III), PI567.583D(MG IV), PI567.286(MG IV), PI567.492(MG IV), and PI567.373B(MG V)
	Class E: > 4.0
13	PI567.510A(MG III), PI567.581(MG IV), PI567.336B(MG IV), PI567.577(MG IV), PI567.421(MG IV), PI567.285(MG IV), PI567.535A(MG IV), PI567.303A(MG IV), PI567.336A(MG IV), PI567.562A(MG IV), PI567.516C(MG IV), PI567.342(MG V), and PI567.568B(MG V)

19 lines was in MG II, 4 were in MG III, 11 in MG IV, and 3 in MG V. The clusters are referred to as A, B, and C. A has 7 members including the cultivar ‘Pharaoh’. B has 10 members, and C has 2 members. In the case of the marker Sat_038, there was amplification for 22 of the 40 lines. These 22 lines and cultivars were grouped into 3 clusters. Cluster A has 2 members, the control cultivar Forrest and one new MG IV line. B consists of 3 lines belonging to MG III and MG IV. C is by far the largest with 17 members coming from all 4 MGs.

Along with their SDS response and their yield in t ha^{-1}, the members of each cluster are presented in Table 4 for SoyABAB and in Table 5 for Sat_038.

Data on these SSR clusters indicate that members in any one given class do not necessarily have similar SDS response, yield or any other agronomically important trait. The cluster in fact rely on different alleles of the same locus or microsatellite, which can be, as in the case of Sat_038, physically linked to SCN race 3 resistance genes in soybean. The detection of more than 1 allele of the same locus would then suggest that the new PI lines already known to be SCN race 3 resistant are also genetically diverse in that regard. Although a total of 31 new lines were evaluated in the field and screened with the two microsatellite markers, only 24 lines and two cultivars are presented in the dendrogram on the basis of banding pattern (Figure 2).

Each of the 24 lines had at least one band for one of the two markers, as presented in Tables 4 and 5. Allelic variation at SSR loci in soybean is very common. The existence of different alleles for the locus SoyABAB was recently discussed Li et al. (2000). They mentioned different band sizes that were much smaller than the predicted 290 bp when wild soybean populations were screened with this marker. This variation could very well explain the different clusters that were found in the present study. In the case of the locus Satt039, a smaller variation in banding pattern was recently reported by Njiti et al. (2002), but only adapted cultivars were used in their study. The dendrogram reveals an overall similarity coefficient larger than 50% for all the lines. Several lines shared over 80% similarity with the cultivar ‘Forrest’ known to be resistant to SDS in the field.

Table 4. Genetic Relatedness by SSR marker SoyABAB

Line	MG	SQRTDX	Yield in t ha^{-1}
		Class A	
PI567.510A	III	5.01	1.41
PI567.491A	III	2.71	2.72
Pharaoh	IV	0.93	4.15
PI567.285	IV	4.65	1.40
PI437.654	IV	3.87	1.30
PI209.332	IV	1.27	0.70
PI567.568B	V	4.04	1.51
		Class B	
PI567.364	II	1.49	1.86
PI567.365	III	1.34	1.55
PI567.336A	IV	4.35	1.00
PI567.535A	IV	4.19	3.04
PI404.166	IV	2.28	0.42
PI567.492	IV	3.16	1.8
PI567.583C	IV	1.66	1.37
PI567.583D	IV	3.96	1.45
PI567.342	V	4.11	0.95
PI567.373B	V	3.23	1.62
		Class C	
PI567.363B	III	1.89	1.80
PI567.636	IV	2.58	0.30

Table 5. Classes of genetic relatedness by SSR marker Sat_038

Line	MG	SQRTDX	Yield in t ha^{-1}
		Class A	
PI567.562A	IV	4.86	2.37
Forrest	V	0.79	3.72
		Class B	
PI567.365	III	1.34	1.55
PI567.285	IV	4.65	1.40
PI567.336A	IV	4.35	1.00
		Class C	
PI567.364	II	1.49	1.86
PI567.491A	III	2.71	2.72
PI567.363B	III	1.89	1.80
PI404.166	IV	2.28	0.42
PI437.654	IV	3.87	1.30
PI567.636	IV	2.58	0.30
PI209.332	IV	1.27	0.70
PI567.583C	IV	1.66	1.37
PI567.492	IV	3.16	1.80
PI567.303A	IV	5.03	2.06
PI567.421	IV	6.01	1.79
PI567.336B	IV	4.56	1.04
Pharaoh	IV	0.93	4.15
PI89.772	IV	3.32	1.15
PI567.568B	V	4.04	1.51
PI567.660B	V	2.71	1.30

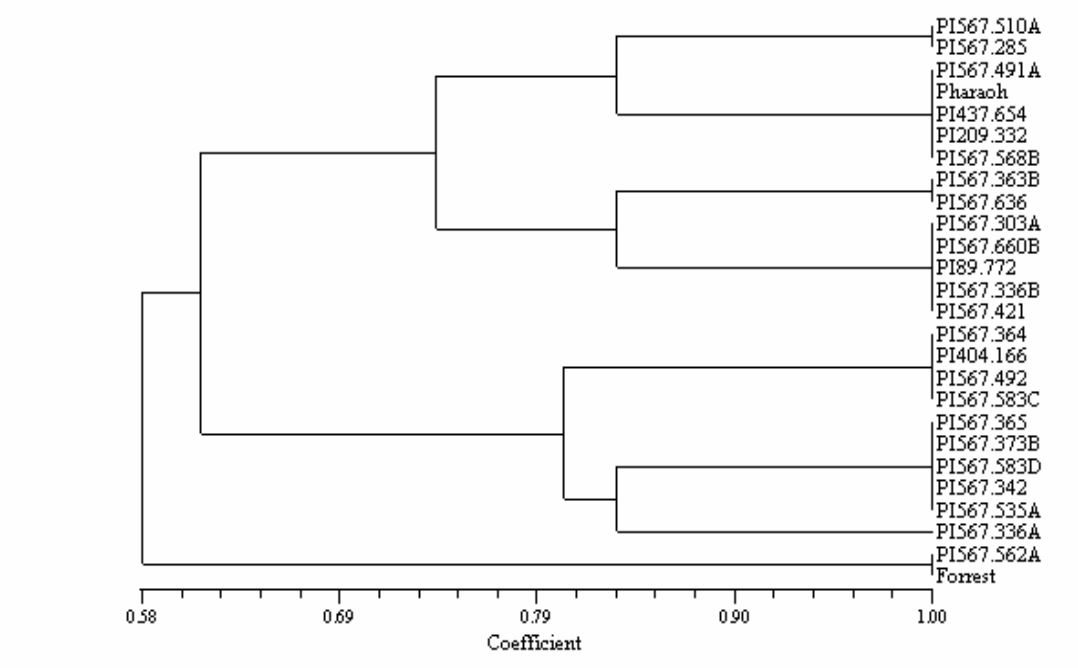

Figure 2. Genetic diversity of the new PI lines as revealed by the microsatellite markers SoyABAB and Sat_038 using the coefficient of similarity obtained with the software NTSYSpc.

Conclusions

The agronomic performance of the new PI lines at 2 locations in southern Illinois has identified excellent candidates for breeding programs. Flower color was evenly distributed between white and purple. Flowering date and harvest maturity were as expected, and generally more days were needed in the later MGs to complete flowering and harvest maturity. Seed yield was generally low but a few MG IV lines yielded better than some controls. Twenty-four of the 31 new lines had a yellow seed coat with a brown hilum. In most old sources, SCN resistance was strongly associated with black seed coat. Whenever these sources were used in breeding programs, numerous backcrosses were needed to break this undesirable linkage, which could increase the cost and time

necessary to develop an improved variety. Because of their yellow seed coat, the new PI lines can be readily used in breeding programs as parents, mainly as SCN resistance sources, and in some instances as new SDS resistance sources as well.

The lines varied greatly in their response to SDS in the field. They were generally susceptible to the disease but some lines were comparable to the SDS resistant controls. Considering that these PI lines are new SCN resistance sources, it is interesting to identify a few lines that had a good SDS response in the field (low SQRTDX), combined with a yellow seed coat. These lines were PI567.507B, PI567.418A, PI567.512B, PI567.364, PI567.365, PI567.363B, PI567.445B, PI567.583C, PI567.415A, PI567.373, and PI567.660B. Further evaluation of these 11 elite lines might help identify potential parents with a combined resistance to SCN and SDS in the field.

Based on banding pattern, the microsatellite marker SoyABAB has separated 19 of the 40 lines into 3 different clusters, each containing both new and old sources. In the case of the marker Sat_038, there was amplification for 22 of the 40 lines screened. These 22 lines and cultivars were also grouped into 3 clusters on the basis of their banding pattern. Some degree of variation was also detected when the banding pattern of the two markers was combined to create a dendrogram for the lines. Although the 31 new PI lines are resistant to SCN, they are also genetically diverse. This genetic variation can be used in a molecular breeding program to identify quantitative trait loci (QTL) associated with SCN and SDS resistance in the field.

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