

## Registration of AR10SDS Soybean Germplasm Partially Resistant to Sudden Death Syndrome and Resistant to Soybean Cyst Nematode

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### ABSTRACT

Disease resistance is an important crop trait that protects yield and farmers' incomes. The objective of the soybean breeding program for disease resistance at Iowa State University (ISU) is to develop germplasm lines for Iowa. AR10SDS (Reg. No. GP-391, PI 669816) is the first sudden death syndrome (SDS) partially resistant line released by ISU, Project nos. 4403 and 0159 (Agronomy and Plant Pathology departments), ISU Research Foundation (ISURF) Docket #03264. AR10SDS is partially resistant to *Fusarium virguliforme* which causes SDS, and resistant to soybean cyst nematode (SCN) phenotypic race 3 (HG types 0 and 7) caused by *Heterodera glycines*. AR10SDS is a bulk of 60 F<sub>3:8</sub> plants uniform in plant and seed traits. The parentage is 'Ripley' × 'IA1008'. Ripley is resistant to SDS. IA1008 is resistant to SCN. The cross and generations advances were made in Puerto Rico beginning in 2000, ISU research site at the Isabela Substation, University of Puerto Rico, Isabela, PR. Yield was evaluated from 2003 to 2006 in Iowa and in the northern U.S. regions. From 2003 to 2011, SDS resistance was evaluated under greenhouse conditions and in SDS-infested field soil. Soybean cyst nematode screening was conducted in 2005 to 2006 under greenhouse conditions. Three SDS resistance quantitative trait loci (QTLs) were inherited from Ripley, and two SCN resistance QTLs from IA1008. Seed yield is similar to IA1008 and significantly ( $P = 0.05$ ) better than 'Parker' and 'Freeborn'. It is of early- to mid-maturity group I and adapted from 40 to 42° N latitude. AR10SDS may serve breeding programs for its dual disease resistance.

GENETIC RESISTANCE to pests is one of the most desirable traits for soybean [*Glycine max* (L.) Merrill] production because it provides protection to yield and income stability to growers. It is also ecologically sustainable. Sudden death syndrome (SDS), caused by the fungus *Fusarium virguliforme*, is a relatively new disease in the northern U.S. soybean production region. It was first observed by H.J. Walters in Arkansas in 1971 and it remained an unnamed curiosity until 1982, when M.C. Hirrel determined that it caused severe yield reductions in that state (Roy et al., 1997). The pathogen colonizes soybean roots, producing root rot (Rupe, 1989). Once established in roots, the pathogen produces toxin(s) that translocate to the aerial portions of the soybean plant and cause foliar symptoms, such as chlorosis and necrosis. Leaf death occurs in extreme cases.

Sudden death syndrome was first observed in Iowa on experimental plots in 1993 (Yang and Rizvi, 1994). In 1994 and 1995, the geographic distribution of the disease in Iowa indicated a decreasing trend of SDS prevalence from east to west (Yang and Lundeen, 1997). The disease was found in commercial production fields in four Iowa crop districts; central, north-central, east-central, and southeastern. At the time, no SDS was observed in western Iowa.

Presently in Iowa, the disease severely reduces soybean yields and has spread to large areas in the state (X.B. Yang, personal communication, Iowa State University, 2004). It has even been identified further north, in soybean production regions of the state of Minnesota (J. Orf, personal communication, University of Minnesota, 2005). During summer 2000, the incidence of the disease in Iowa was so prevalent that the Iowa State University (ISU) breeding project for disease and pest resistances made the first crosses to develop populations to select for SDS resistance.

Soybean cyst nematode (SCN), caused by *Heterodera glycines* Ichinohe, is another yield deterrent to soybean, causing important economic losses to the commodity (Wrather and

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Journal of Plant Registrations 8:200–210 (2014).  
doi: 10.3198/jpr2012.10.0046crg  
Received 18 Oct. 2012. Registration by CSSA.  
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**Abbreviations:** DI, disease incidence; DS, disease severity; DX, disease index; Gm, *Glycine max*; HG, *Heterodera glycines*; ISU, Iowa State University; ISURF, Iowa State University Research Foundation; LG, linkage group; MG, maturity group; PCR, polymerase chain reaction; QTLs, quantitative trait loci; SCN, soybean cyst nematode; SDS, sudden death syndrome; SIU, Southern Illinois University.

Koenning, 2006). A soil survey conducted in Iowa in 2008 indicated that the nematode was present in 99% of the counties of the state ([www.plantpath.iastate.edu/scn/](http://www.plantpath.iastate.edu/scn/)). The nematode attacks soybean roots, causing great disruption to plant nutrient absorption and to the biology of the soybean plant. A review of the last 10 yr of the soybean cyst nematode variety trials conducted at ISU by the Extension Service revealed that the *Heterodera glycines* (HG) type (Niblack et al., 2002) equivalent to SCN race 3 was prevalent in 48 of the 89 experimental sites, which represented 54% of the sites (G. Tylka, personal communication, ISU, 2010).

In soils where *F. virguliforme* and *H. glycines* are present, synergistic effects on soybean yields are observed (Gelin et al., 2006), making it a necessity to release improved germplasm lines possessing genetic resistance to both pathogens. This was the objective of the ISU soybean breeding project in releasing AR10SDS (Reg. No. GP-391, PI 669816), which is partially resistant to SDS and resistant to SCN. AR10SDS is the first line developed by the ISU project released on the basis of its resistance to SDS. Seed yield of AR10SDS is similar to the cultivar IA1008 (Cianzio and Fehr, 1999), one of the parents of the line, and significantly ( $P = 0.05$ ) better than 'Parker' (Orf, and Kennedy, 1994) and 'Freeborn' (Orf, and Young, 1997). It is of early- to mid maturity group (MG) I and adapted to latitudes of 40 to 42° N.

## Materials and Methods

### Pedigree

AR10SDS (experimental line AR03-163008) is an  $F_3$ -derived  $F_8$  bulk from the cross of 'Ripley' × IA1008. Ripley (Cooper et al., 1990) is a high-yielding, lodging-resistant determinate cultivar, jointly released by the USDA-ARS and the Ohio Agricultural Research and Development Center in 1985. Ripley is of MG IV and resistant to SDS. It was derived from the cross of 'Hodgson' × V68-1034. Hodgson (Lambert and Kennedy, 1975) was released in 1975. V68-1034 (G. Buss, personal communication, Virginia Tech. University, 2000) is an experimental line selected from the cross of 'York' × PI 71506. York is of MG V and was developed by the Virginia Agricultural Experiment Station (Smith, 1968). PI 71506 is a plant introduction from the National Soybean Germplasm Collection (<http://www.ars-grin.gov/>). IA1008 was selected from the cross of 'NK20-20' × 'Jack' and released by ISU. IA1008 is of MG I and resistant to SCN race 3. NK20-20 is a cultivar developed by the Northrup King Co., Minneapolis, MN. Jack (Nickell et al., 1990) was developed by the University of Illinois, Urbana, IL, and its SCN resistance traces to PI 88788, a plant introduction from the National Soybean Germplasm Collection (<http://www.ars-grin.gov/>).

### Line Development and Evaluation of Traits

The cross of Ripley × IA1008 was made in Puerto Rico in June 2000, at the ISU research site located at the Isabela Substation of the University of Puerto Rico, Isabela, PR. The cross designated as AX17925 was part of a group of crosses made with the objective to develop high-yielding lines with resistance to SDS. Seven  $F_1$  seeds were obtained for AX17925. During October 2000,  $F_1$  plants were obtained, and morphological markers (flower and pubescence colors) were used to confirm the hybrid nature of the  $F_1$  plants. After confirmation of the hybrid nature of the  $F_1$

plants, the  $F_2$  seed was harvested in bulk and planted in Puerto Rico in February 2001. The  $F_3$  was harvested by sampling one seed from each  $F_2$  plant. A second sample similarly harvested was maintained in cold storage as reserve. After development of the line was completed, the reserve seed was discarded.

In summer 2001, the  $F_3$  seed was planted at Brunner Farm, near Ames, IA, with the objective to harvest  $F_3$  individual plants classified by maturity, forming the  $F_3$ -derived  $F_4$  lines. During fall 2001 and the winter 2002, the  $F_{3,4}$  lines were screened for SDS under greenhouse conditions at the Cianzio laboratory, Agronomy Department Research Greenhouses, at Ames. The screening was conducted to discard all  $F_{3,4}$  lines from the population that were susceptible to SDS. The screening protocol is described in the following section.  $F_{3,4}$  lines resistant to SDS were identified from these tests.

In summer 2002, the remnant seed of the  $F_{3,4}$  lines that had been screened for resistance to SDS were evaluated in the first yield test at one central location, near Ames. The yield and agronomic evaluations were conducted in short one-row plots, 63 cm long, planted at 8 seed per 30 cm<sup>-1</sup>, with two replications. Lines and control cultivars were randomized in a randomized complete block design. Agronomic data, namely plant height, lodging, and maturity date, along with seed yield were recorded. All individual plots were harvested and the superior yielding lines were selected. The selected lines were screened for SDS resistance during the fall and winter of 2002–2003, following protocols described in the following section.

During summer 2003, the  $F_{3,5}$  lines resistant to SDS and with superior yield were evaluated in replicated tests on non-SDS infested soil at two Iowa locations, Sutherland and Kanawha, in the second yield tests. In summer 2004, the experimental line AR03-163008 was evaluated in the third yield tests also in Iowa at two locations, Mason City and New Hampton. Two-row plots were used each year. Also during summer 2004, the SDS resistance of the line was evaluated at the SDS Regional Tests, conducted under greenhouse conditions at Southern Illinois University (SIU), Carbondale, IL (J. Bond, personal communication, SIU, 2004). Since 2007, AR10SDS has been used as a check genotype in the SDS Regional Tests, conducted at SIU, Carbondale (J. Bond, personal communication, SIU, 2007).

During summers 2005 and 2006, AR03-163008 ( $F_{3,7}$  and  $F_{3,8}$  lines, respectively) was evaluated in the Northern Regional Soybean Cyst Nematode Tests, in replicated 4-row plots, at several locations on SCN-infested soil (T. Cary, personal communication, University of Illinois, 2005 and 2006). The row length and seeding rate varied each year and at each location depending on the collaborator planting the test.

### Sudden Death Syndrome Disease Screening and Disease Assessment

#### Screening for Sudden Death Syndrome Resistance under Greenhouse Conditions

Screening tests were conducted at the Cianzio laboratory in 2004 (Table 1). Greenhouse SDS screening protocols were developed by X.B. Yang (personal communication, ISU, 2001), modified by P. Lundeen (personal communication, ISU, 2002), and later patented by D. Lightfoot (2007) at SIU. The patented screening test was used with permission. Styrofoam cups (240

mL) with three holes in the bottom were filled with 150 mL of 2:1 sand:soil mixed with 12 mL of ground sorghum [*Sorghum bicolor* (L.) Moench], inoculum and a second layer of 30 mL of sand:soil mixed (no inoculum). Five soybean seeds of each genotype were placed in a 240-mL Styrofoam cup and covered with an additional 30 mL of sand:soil. Cups were maintained on the greenhouse bench at 20°C. Plants were watered once daily to maintain soil moisture. Three replications per genotype were evaluated. The light bulbs used were 400 W High Pressure Sodium, model Sylvania 67533-LU400/ECO-HPS.

Three isolates of *F. virguliforme*, Clinton 1B from Clinton County, IA, and two isolates from Scott County, IA, Scott F2111a and Scott B2, were obtained from roots of SDS symptomatic plants from production fields. Isolates were grown on one-third strength potato dextrose agar without antibiotics for 5 wk at room temperature in the laboratory. Plates were placed in a drawer in the dark, and/or on the countertop and covered. Five hundred grams of white sorghum seeds were soaked overnight in water in 2-quart mason jars, covered with plastic lids, autoclaved for 1 h on two consecutive days, and inoculated with 5 mycelia plugs (10 mm diameter) of Clinton and Scott isolates of *F. virguliforme*. Jars were incubated at room temperature for 4 wk and shaken every 3 to 4 d for 1 to 2 min for the first 2 wk to ensure uniform fungal growth. To simulate Iowa natural photoperiod conditions in the greenhouse, artificial light bulbs were on for 15 h.

In the greenhouse, SDS symptoms were evaluated at 35 d after planting, vegetative stages V3 to V4 (Fehr et al., 1971). Disease incidence (DI), disease severity (DS), and disease index (DX) were recorded (Bond and Schmidt, 2005). Disease incidence is the percentage of plants in pots showing leaf symptoms. Disease

severity was scored on a 1-to-9 scale over all plants in a cup, where 1 = 1 to 10% of the leaf surface chlorotic; 2 = 11 to 20% of the leaf surface chlorotic or 6 to 10% necrotic; 3 = 21 to 40% of the leaf surface chlorotic or 11 to 20% necrotic; 4 = 41 to 60% of the leaf surface chlorotic or 21 to 40% necrotic; 5 = >60% of the leaf surface chlorotic or >40% necrotic; 6 = <one-third premature defoliation; 7 = one-third to two-thirds premature defoliation; 8 = >two-thirds premature defoliation; and 9 = plant death before normal defoliation due to senescence. Disease index is the ratio of  $DI \times DS/9$ . Number of plants per cup was also recorded.

### Screening of Sudden Death Syndrome Resistance in Field Tests

Sudden death syndrome evaluations were conducted in the field in Iowa where irrigation was available and SDS had been previously observed and recorded. The location was the Hinds Farm Experimental Research Station, near Ames. A randomized complete block design with three replications was used in each experiment. Plantings were conducted on plots artificially inoculated with SDS.

The inoculum was prepared at the Cianzio laboratory using sorghum seed inoculated with a mixture of two isolates of *Fusarium virguliforme* obtained from naturally infested SDS fields in Iowa (P. Lundeen, personal communication, ISU). Five-week-old inoculum was sieved to a maximum size of 0.06 mm (1/4 inch), then placed in each of the soybean seed packets 48 h before planting. The amount of 4 cm<sup>3</sup> was used per 30 cm (1 ft.) of row in each of the two-row plots, 2.40 m (8 ft.) long. The DS scale in the field is the average percentage of total foliar surface lost to necrosis/chlorosis on plants with symptoms on a plot basis. It was rated as 1 = 1 to 10% of the leaf surface chlorotic or 1 to 5% necrotic; 2 = 11 to 20% of the leaf surface chlorotic or 6 to 10% necrotic; 3 = 21 to 40% of the leaf surface chlorotic or 11 to 20% necrotic; 4 = 41 to 60% of the leaf surface chlorotic or 21 to 40% necrotic; 5 = >60% of the leaf surface chlorotic or >40% necrotic; 6 = <one-third premature defoliation; 7 = one-third to two-thirds premature defoliation; 8 = >two-thirds premature defoliation; and 9 = plant death before normal defoliation due to senescence.

In the field tests, plots were rated when the majority of the plants in the lines were at the R6 (Fehr et al., 1971) reproductive growth stage. Disease incidence and DS were recorded on a plot basis, and DX was calculated (Table 2) (Bond and Schmidt, 2005). In all SDS screening tests, susceptible and resistant genotypes and control cultivars were included. Control genotypes were the same as used in the SDS Soybean Variety Tests performed at SIU.

### Soybean Cyst Nematode Screening

Data reported for SCN were obtained from nematode screenings conducted on all genotypes entered in the SCN Uniform Tests (Table 3). Screenings were conducted at Purdue University (W. Crochet, personal communication, USDA, Purdue-ACRE, 2005) and at the University of Illinois (T. Niblack personal communication, Ohio State University, 2005). Each SCN test was conducted using a randomized complete block design with three replications.

The test at Purdue, IN, was done by bringing in SCN-infested soil into the greenhouse and using it to test each soybean line for resistance to the SCN population identified in that field

**Table 1. Sudden death syndrome (SDS) ratings of AR10SDS and of resistant and susceptible controls at Ames, IA, in 2004.**

Experimental line and control <sup>†</sup>	Greenhouse inoculated <sup>‡</sup>		
	Disease incidence <sup>§</sup>	Disease severity <sup>¶</sup>	Disease index <sup>#</sup>
	%	1–9	
AR10SDS	3.3	1.7	0.9
Resistant controls			
MN1606SP	5.0	4.0	2.2
Venus RR	10.0	5.3	6.3
Susceptible controls			
5171 RR	8.3	4.7	4.4
C17122 RR	20.0	4.7	9.6

<sup>†</sup> Genotypes used as controls in the SDS Regional Variety Test, conducted at Southern Illinois University, Carbondale (J. Bond, personal communication, Southern Illinois University, 2004). Control genotypes with RR = Round-Up Ready trait.

<sup>‡</sup> Artificially inoculated greenhouse tests.

<sup>§</sup> Disease incidence (DI): percentage of plants in plot (pot) showing leaf symptoms.

<sup>¶</sup> Disease severity (DS): 1 = 1 to 10% of the leaf surface chlorotic or 1 to 5% necrotic, 2 = 11 to 20% of the leaf surface chlorotic or 6 to 10% necrotic, 3 = 21 to 40% of the leaf surface chlorotic or 11 to 20% necrotic, 4 = 41 to 60% of the leaf surface chlorotic or 21 to 40% necrotic, 5 = >60% chlorotic or >40% necrotic, 6 = up to one-third premature defoliation, 7 = one-third to two-thirds premature defoliation, 8 = <two-thirds premature defoliation, and 9 = plant death before normal defoliation due to senescence. It was rated only on plants showing symptoms.

<sup>#</sup> Disease index (DX) = (DI × DS)/9.

(W. Crochet, personal communication, USDA, Purdue-ACRE, 2005). Field SCN populations used at Purdue were classified using the race system (Schmitt and Shannon, 1992). The SCN genotype reactions were classified as R = resistant, S = susceptible, MR = moderately resistant, and MS = moderately susceptible. The female index was not provided in this test.

Tests conducted at the University of Illinois were done using sterilized sandy soil artificially inoculated with 1000 eggs (T. Niblack, personal communication, Ohio State University, 2005). The SCN populations were classified using the HG type as defined by Niblack et al. (2002). The SCN reactions for tests conducted at Urbana, IL, were classified as HR = highly resistant, R = resistant, MR = moderately resistant, LR = low

**Table 2. Sudden death syndrome (SDS) disease index ratings of AR10SDS and resistant and susceptible controls in the Soybean Sudden Death Syndrome Regional Tests, 2004 to 2005 and 2007 to 2011, Southern Illinois University, Carbondale, IL (data used with permission).**

Experimental line and cultivars†	SDS disease index rating‡					
	2004					
	Carbondale, IL§	Ames, IA	Crawfordsville, IA	Harrow, ON	Paris, IL¶	Ursa, IL¶
AR10SDS	2.8	1	9	1	1	16
MN1606SP (R)	2.2	2	0	1	0	8
Venus RR (MR)	1.1	6	2	1	3	2
Myc 5171 (MS)	4.5	4	8	0	15	20
LSD (0.05)#	–	9	NS	–	6	13
	2005					
	Paris, IL¶	Pontiac, IL¶	Ames, IA	Harrow, ON¶		
AR10SDS	0	0	0	14		
MN1606SP (R)	0	1	0	26		
Venus RR (MR)	0	0	0	15		
Myc 5171 (MS)	2	20	0	19		
M97-357138(S)	10	12	9	27		
LSD (0.05)#	5	6	NS	18		
	2007					
	Waseca, MN¶	Pontiac, IL	Paris, IL	Urbana, IL¶		
AR10SDS	2	0	0	1		
M00-456052 (R)	–	–	–	–		
MN1606SP (R)	10	0	0	0		
Myc 5171 (MS)	20	8	4	17		
M97-357138 (S)	0	0	4	3		
LSD (0.05)#	NS	2	NS	NS		
	2008					
	Havana, IL¶	Kanawha, IA				
AR10SDS	1	0				
M00-456052 (R)	2	1				
MN1606SP (R)	2	4				
Myc 5171 (MS)	4	3				
M97-357138 (S)	14	8				
LSD (0.05)#	9	0				
	2009					
	Kanawha, IA	Champaign, IL¶	Harrow, ON¶	Waseca, MN		
AR10SDS	0	1	0	9		
M00-456052 (R)	0	1	0	0		
MN1606SP (R)	0	1	0	1		
Myc 5171 (MS)	1	13	19	6		
M97-357138 (S)	5	27	33	8		
LSD (0.05)#	4	46	7	14		
	2010					
	Kanawha, IA	Champaign, IL¶	Harrow, ON¶	Waseca, MN		
AR10SDS	2	0	10	1		
M00-456052 (R)	6	0	0	0		
MN1606SP (R)	5	1	7	2		
Myc 5171 (MS)	49	1	44	2		
M97-357138 (S)	49	23	31	3		
LSD (0.05)#	17	8	3	7		



Table 2. Continued.

Experimental line and cultivars†	SDS disease index rating‡				
	2011				
	Harrow, ON¶	Manito, IL¶	Kanawha, IA	Waseca, MN	Rosemount, MN¶
AR10SDS	3	0	0	0	2
M00-456052 (R)	25	4	0	0	2
MN1606SP (R)	3	5	0	1	0
Venus RR (MR)	34	22	1	3	10
Myc 5171 (MS)	57	36	0	0	3
M97-357138 (S)	62	23	0	0	0
LSD (0.05)#	NS	17	4	NS	NS

† R = resistant; MR = moderately resistant; MS = moderately susceptible; S = susceptible; RR = Round-up Ready trait.

‡ Disease index = (Disease Incidence × Disease Severity)/9. Disease incidence = percentage of plants showing disease symptoms. Disease severity = average over all plants in the plot with disease on a scale of 1 to 9, where 1 = 1 to 10% of the leaf surface chlorotic or 1 to 5% necrotic, 2 = 11 to 20% of the leaf surface chlorotic or 6 to 10% necrotic, 3 = 21 to 40% of the leaf surface chlorotic or 11 to 20% necrotic, 4 = 41 to 60% of the leaf surface chlorotic or 21 to 40% necrotic, 5 = >60% chlorotic or >40% necrotic, 6 = up to one-third premature defoliation, 7 = one-third to two-thirds premature defoliation, 8 = >two-thirds premature defoliation, and 9 = plant death before normal defoliation due to senescence.

§ Carbondale, IL, 2004 greenhouse test, disease severity reported.

¶ Disease index reported.

#LSD not provided by cooperators in the Soybean Sudden Death Syndrome Regional Tests.

resistant, NR = no effective resistance (Niblack et al., 2002). In these tests, the female index was provided and was calculated for each entry by dividing the mean number of cysts on the genotype by the mean number of cysts on the susceptible cultivar Lee 74 (Caviness et al., 1975), multiplied by 100.

## Yield Tests and Seed Traits

During 2003 and 2004, AR10SDS was evaluated in Iowa at each of two locations in a randomized complete randomized block design with two replications (Table 4). Two-row plots were used. Each row was 3.8 m long and planted with 30 seed cm<sup>-1</sup> at each location. During 2005 and 2006, the line was evaluated at

the SCN Regional Uniform Tests on SCN-infested soils (Table 5). The tests were planted at multiple locations each year in a randomized complete block design, with varying four-row plot sizes, depending on location and year. Three replications were used at each location. In 2005, five locations were planted. Eight locations were planted in 2006.

## Statistical Analysis

SAS statistical software (SAS Institute, 2000) was used to run ANOVAs and Fisher's protected LSDs for plot data. Genotype × environment was used to estimate error variances for traits in the SCN regional trials (P. Dixon, personal communication,

Table 3. Average soybean cyst nematode (SCN) ratings of AR10SDS and cultivars in greenhouse conditions. All tests were replicated. The data were extracted from the 2005 and 2006 Northern Regional Soybean Cyst Nematode Tests, University of Illinois, Department of Crop Sciences, Urbana, IL (data used with permission).

Experimental line and cultivars†	IN greenhouse SCN screening‡				IL greenhouse SCN screening§			
	2005							
	Race 3 Pulaski	Race 1 Jasper	Race 1 Tippecanoe		Race 3 HG Type 7		Race 1 HG Type 2.5.7	
Reaction				FI	Reaction	FI	Reaction	
AR10SDS	R	S	MR		4	HR	73	NR
Parker	MS	S	S		43	LR	87	NR
IA1008	R	MS	MR		5	HR	65	NR
Freeborn	MR	MS	MR		1	HR	31	MR
IAR2001 BSR	MR	S	S		41	LR	54	LR
2006								
	Race 6 Tippecanoe	Race 1 Tippecanoe	Race 5 White	Race 3 Vigo	Race 3 HG Type 7		Race 1 HG Type 2.5.7	
Reaction				FI	Reaction	FI	Reaction	
AR10SDS	R	MS	R	R	10	R	89	NR
Parker	MS	MS	MS	MS	73	NR	89	NR
IA1008	R	R	R	R	9	HR	53	LR
IA1021	S	S	S	MS	56	LR	56	LR
Freeborn	R	R	R	R	4	HR	67	NR

† IA1008, and Freeborn are SCN resistant; Parker and IA1021 are SCN susceptible.

‡ R = resistant; S = susceptible; MR = moderately resistant; MS = moderately susceptible; female index (FI) not provided.

§ FI = female index; HR = highly resistant; R = resistant; MR = moderately resistant; LR = low resistant; NR = no effective resistance.

**Table 4. Average seed yield and agronomic performance of AR10SDS, the soybean cyst nematode-resistant parent IA1008, and check cultivars planted on non sudden death syndrome -infested soil in two replications of two-row plots at two locations in Iowa in 2003 and 2004.**

Experimental line and cultivars	Individual locations		Average over locations			
	Seed yield		Maturity date	Lodging score†	Plant height	
	kg ha <sup>-1</sup>					
			<b>2003</b>			
	<b>Curtiss Farm</b>	<b>Kanawha</b>				
AR10SDS	2360.0	3239.5	2800	18 Sept.	2.0	89
IA1008‡	2002.5	2420.5	2212	13 Sept.	2.3	92
IA1006	1243.5	2617.5	1931	16 Sept.	2.0	85
Parker	2234.0	2778.0	2506	19 Sept.	1.5	88
LSD (0.05)	413.6	325.6	254.1		0.51	7.4
			<b>2004</b>			
	<b>Mason City</b>	<b>New Hampton</b>				
AR10SDS	3794.0	3333.0	3564	22 Sept.	2.3	86
IA1008‡	3562.5	3421.0	3492	18 Sept.	1.5	89
IA2068	3236.5	3387.0	3312	14 Sept.	2.0	81
IA2052	3320.5	3058.5	3190	21 Sept.	1.8	84
Parker	3288.0	3330.0	3309	15 Sept.	2.8	96
LSD (0.05)	400.4	333.2	249.6		0.59	7.2

† 1 = almost all plants erect, 2 = all plants leaning slightly or a few plants down, 3 = all plants leaning moderately (45 degrees) or 25 to 0% of the plants down, 4 = all plants leaning considerably or 50 to 80% of the plants down, 5 = all plants down or prostrate.

‡ IA1008 is one of the parents of AR10SDS.

ISU, 2009). Fisher's protected LSDs were calculated for regional yield trials by summing individual location estimates of error variances from each location CV. The estimated variance was then used to calculate Fisher's protected LSD. The LSDs for all traits evaluated in Iowa tests were calculated using a standard ANOVA analysis.

For the SDS disease index, a combined LSD across locations and years was not calculated because of the highly variable disease levels across locations and years. Individual location LSDs, calculated by the different state cooperators and submitted with the averages, were listed where disease scores were significant (Table 2). Seed traits were scored by bulking

**Table 5. Average seed yield and agronomic performance of AR10SDS and cultivars tested in the northern region of the United States in 2005 and 2006. All tests were replicated and planted on soybean cyst nematode (SCN)-infested soil. Plots were four rows of different sizes depending on locations. The data were extracted from the 2005 and 2006 Northern Regional Soybean Cyst Nematode Tests, University of Illinois, Department of Crop Sciences, Urbana, IL (data used with permission).**

Experimental line and cultivars†	Yield		Maturity date 1 Sept. = Day 1	Lodging score‡	Plant height
	Infested	Rank			
	kg ha <sup>-1</sup>				
			<b>2005</b>		
			<b>Total of 18 Entries</b>		
<b>No. of tests: 5</b>			<b>7</b>	<b>7</b>	<b>7</b>
AR10SDS	3613	7	21 Sept.	2.0	86
IA1008	3546	10	18 Sept.	1.7	86
Parker	3118	18	16 Sept.	2.2	78
Freeborn	3209	15	15 Sept.	1.6	74
IAR2001 BSR	4025	3	21 Sept.	1.3	76
LSD (0.05)	338.0		1.5	0.32	4.5
			<b>2006</b>		
			<b>Total of 19 entries</b>		
<b>No. of tests: 8</b>			<b>6</b>	<b>8</b>	<b>5</b>
AR10SDS	3430	12	22 Sept.	1.8	97
Parker	2810	19	15 Sept.	1.9	94
IA1008	3496	10	21 Sept.	1.6	102
IA1021	3171	15	17 Sept.	1.5	84
Freeborn	2991	17	17 Sept.	1.6	86
LSD (0.05)	225.1		1.4	0.19	4.4

† IA1008, and Freeborn are SCN resistant; Parker is SCN susceptible; IAR2001 BSR is a cultivar released by the Iowa State University Research Foundation in 2007 that is resistant to brown stem rot (BSR) caused by *Phialophora gregata*.

‡ 1 = almost all plants erect, 2 = all plants leaning slightly or a few plants down, 3 = all plants leaning moderately (45 degrees) or 25 to 0% of the plants down, 4 = all plants leaning considerably or 50 to 80% of the plants down, 5 = all plants down or prostrate.

six individual samples per genotype; therefore, no statistical analysis was conducted (Table 6).

## Molecular Analysis

Molecular analysis was conducted in experimental line AR03-163008, later identified as AR10SDS, to detect the presence of the SDS and SCN resistance quantitative trait loci (QTLs) (Fig. 1–2). A polymerase chain reaction (PCR)–based molecular marker analysis was conducted. Seed of AR03-163008 (AR10SDS) were planted in the greenhouse, and leaf samples were collected for DNA extraction and analysis.

Genomic DNA was isolated according to the CTAB extraction method (CIMMYT, 2005). DNA was isolated from leaf samples of three plants separately for each genotype, the experimental line and the parents. Each of the three samples per genotype was treated as a replication. The final DNA pellet was resuspended in 300  $\mu$ L of 1 $\times$  TE buffer pH 8.0 and stored at  $-20^{\circ}\text{C}$  for further use. DNA quality was checked by running 2  $\mu$ L of the DNA on 1% (w/v) agarose gel. Also, DNA quantity was estimated by running a 1-Kb DNA ladder (NEB Inc.), along with the genomic DNA (not shown). The DNA was diluted with sterile water, and 20 ng was used as a template for the 10  $\mu$ L of PCR run. The PCR amplification was performed using a thermal cycler program of 2 min at  $94^{\circ}\text{C}$ , 38 cycles of denaturation at  $94^{\circ}\text{C}$  for 30 s, annealing at  $50^{\circ}\text{C}$  for 30 s, and extension at  $72^{\circ}\text{C}$  for 1 min. A 10-min extension at  $72^{\circ}\text{C}$  followed the last cycle. The PCR reaction mixtures included 2 mM of  $\text{MgCl}_2$  (Bioline), 0.25  $\mu\text{M}$  each of forward and reverse primers (Integrated DNA Technologies, Inc.), 2  $\mu\text{M}$  deoxy-nucleotide triphosphate and 0.5 U Choice Taq DNA Polymerase (Denville Scientific, Inc.).

Five SDS QTLs identified in Ripley as associated with resistance were obtained from the published literature. The five QTL belong to different linkage groups (LG): LG L, *Glycine max* (Gm) chromosome 19; LG O, Gm10; LG D2, Gm17; LG A2, Gm08; and LG N, Gm03. The markers located on LG L (qFDS002-03), are Satt156, Satt166, and Satt448 (Neto et al., 2007). Markers Satt188 and Satt262 are linked to the LG O QTL (T.I. Pruski, unpublished data, B. W. Diers laboratory, University of Illinois). The markers linked to QTL on LG D2 (cqSDS001), are Satt311 and Satt226 (Neto et al., 2007). Satt187 is linked to the QTL on LG A2 (qFDS003–06), whereas marker Satt631 is associated with the QTL on LG N (qSDS002) (Hashmi, 2004; Kazi et al., 2008).

The molecular markers for the two SCN-resistance QTL specific to the SCN resistant parent IA1008 (resistance donor

PI 88788) located on LG J (Gm 16) and LG G (Gm 18) were designed in the Cianzio laboratory on the basis of previous publications (Chang et al., 2011; Glover et al., 2004). The markers Satt244 and Satt547 are linked to LG J (Qscn3–3), while marker Satt275 is associated with the QTL that belongs to LG G (Qscn3–2). Sequences of the molecular markers were obtained from the “Soybase” database (<http://www.soybase.org/>).

The PCR was done in MyCycler (BioRad Inc.). The amplified products were resolved on a 4% agarose gel along with a 100-bp DNA ladder (NEB Inc.), by running at 150 V for 7 h. The ethidium bromide–stained PCR products were visualized following illumination with an ultraviolet light and photographed in a gel image system.

## Seed Purification

Seed purification of AR10SDS was initiated in the summer of 2006 at the Agronomy Research Farm, near Ames. Sixty  $F_{3,8}$  individual plants of uniform plant height and agronomic and seed traits were individually harvested. During winter 2007, seed derived from each of the 60 plants at the  $F_{3,8}$  generation was checked for hilum color before bulking the  $F_9$  seed for release.

## Characteristics Sudden Death Syndrome Disease Resistance

Throughout the history of its development and in all SDS screening tests, AR10SDS performed mostly as a resistant SDS genotype in the majority of the years tested, from 2004 to 2011 (Tables 1 and 2). Under greenhouse conditions at Ames, in 2004, AR10SDS had better scores than the resistant controls based on each of the three disease assessment criteria; DI, DS, and DX (Table 1). Results were slightly different in the evaluations reported in the Soybean Sudden Death Syndrome Regional Tests in 2004 (Table 2). At some of the locations, AR10SDS had SDS scores comparable to the resistant controls, showing at times superior resistance to controls. Nevertheless, AR10SDS performed consistently worse than the resistant controls in Ursa, IL, and Crawfordsville, IA. In 2005, DX scores of the line were comparable to resistant controls in the test. Similar results were also reported in the SDS tests conducted from 2007 to 2011, in which the germplasm line had disease scores comparable to the resistant control MN1606SP in most cases, except for the 2009 screening in Waseca, MN, and Harrow, ON, in 2010. It could

**Table 6. Average seed size (weight), seed quality, and seed composition of AR10SDS and its parents determined at the Iowa State University Grain Quality Laboratory.†**

Experimental line and parents	Seed					
	Weight	Quality	Protein	Oil	Fiber	Carbohydrates
	g 100 seed <sup>-1</sup>	1–5‡	g kg <sup>-1</sup>			
AR10SDS	17.1	3	370	185	46	219
Parents§						
Ripley	11.7	2	342	172	50	256
IA1008	17.0	3	364	175	48	233

† Estimates of errors not available, the six replications were bulked prior evaluation.

‡ 1 = very good, 2 = good, 3 = fair, 4 = poor, 5 = very poor. Seed quality considers amount and degree of seed coat wrinkling, defective seed coat, greenishness, and moldy or rotten seed. Threshing or handling damage is not included, nor is mottling or other pigment.

§ Ripley is resistant to sudden death syndrome; IA1008 is susceptible to sudden death syndrome and resistant to soybean cyst nematode.

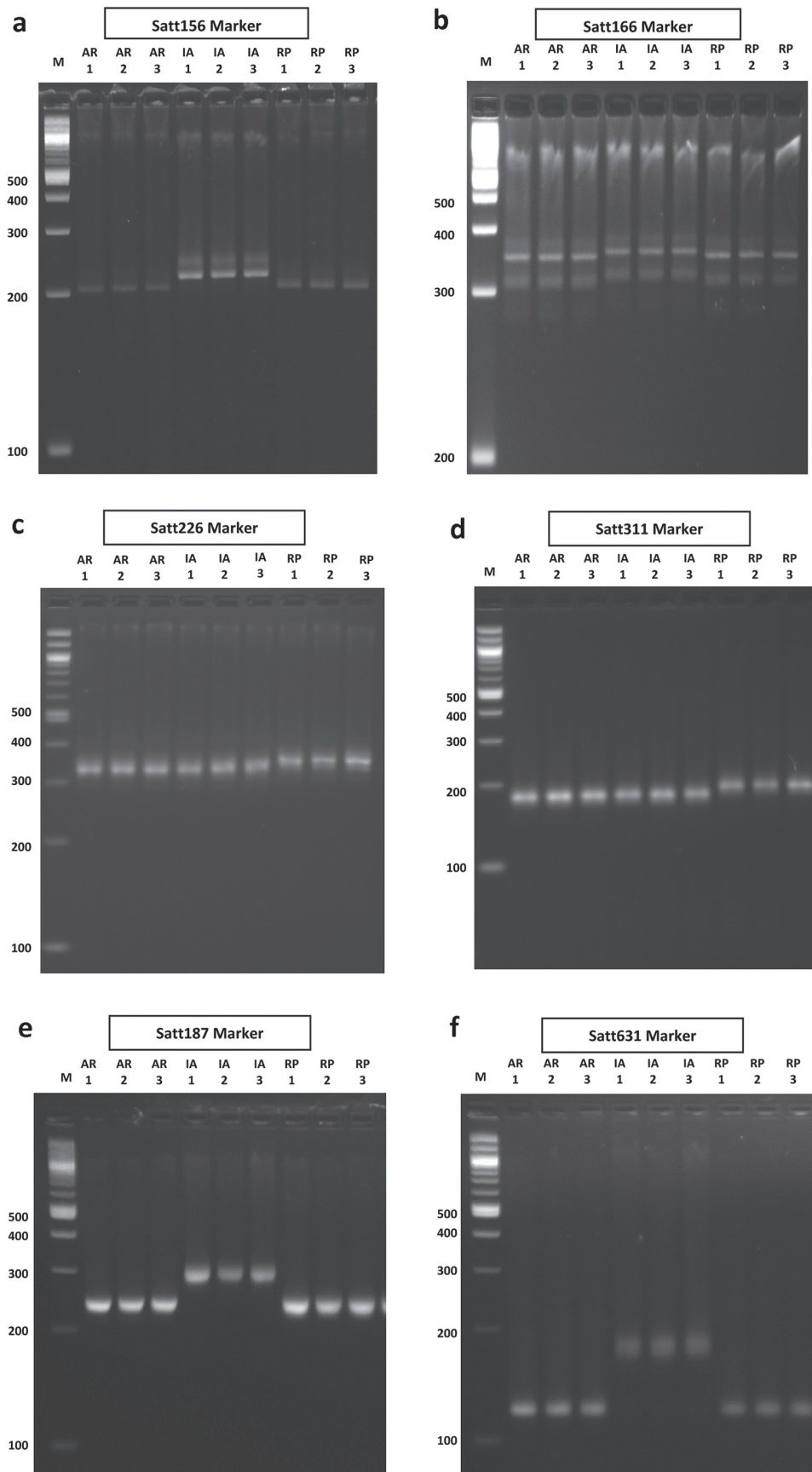


Fig. 1. Agarose gel photographs of PCR products of AR10SDS (AR1, AR2, AR3), IA1008 (IA1, IA2, IA3) and Ripley (RP1, RP2, RP3) with different molecular markers. Three replications were included for each genotype. 100 bp DNA ladder (M) from New England Biolabs was loaded in each gel to show the size of the PCR products. Markers (a) Satt156, (b) Satt166, (c) Satt226, (d) Satt311, (e) Satt187, and (f) Satt631 are associated with SDS resistance and were polymorphic on AR10SDS.



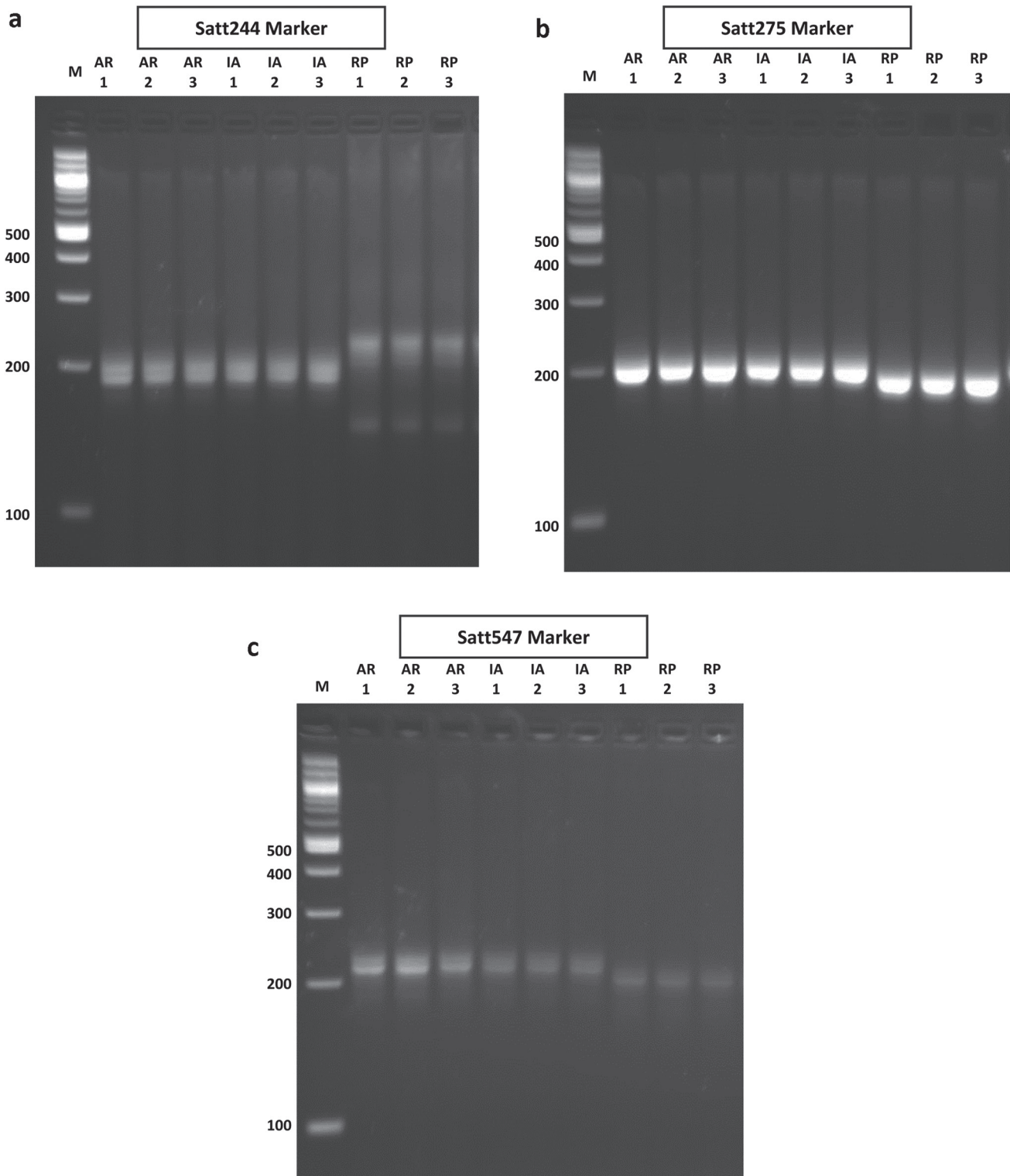


Fig. 2. Agarose gel photographs of PCR products of AR10SDS (AR1, AR2, AR3), IA1008 (IA1, IA2, IA3) and Ripley (RP1, RP2, RP3) with different molecular markers. Three replications were included for each genotype. 100 bp DNA ladder (M) from New England Biolabs was loaded in each gel to show the size of the PCR products. Markers (a) Satt244, (b) Satt275, and (c) Satt547 are associated with SCN resistance and were polymorphic on AR10SDS.

be that differences in resistance scores observed for AR10SDS are attributable to the particular environmental conditions occurring at the location-year combination, interacting with the genotype of the germplasm line.

## Soybean Cyst Nematode Resistance

SCN screening results indicate that AR10SDS was resistant to phenotypic race 3, both in 2005 and 2006, in greenhouse tests conducted at Indiana (Table 3). In 2006, AR10SDS was also resistance to SCN races 5 and 6 according to the test conducted in IN. When AR10SDS was screened at Illinois using sterile soil

to which 1000 eggs counts were added, and for which HG Type was determined (Table 3), it did not have effective resistance to race 1, HG Type 2.5.7. Because *H. glycine* populations in natural soils are heterogeneous and individual nematodes may also be heterozygotes, the differences in resistance reaction to SCN may be explained by the genetic variability among the cyst populations (Niblack et al., 2002).

## Yield and Agronomic Performance

AR10SDS was evaluated at each of two locations in 2003 and 2004 in replicated tests in Iowa (Table 4). AR10SDS yield averaged over the two locations planted in 2003 was significantly ( $P = 0.05$ ) higher than the parent IA1008, and also higher than the checks IA1006 (W.R. Fehr, personal communication, ISU, 1997) and Parker. In 2004, mean yield of AR10SDS was not significantly different from the IA1008 parent, although it was higher in absolute value. During 2005 and 2006, the line was evaluated at the SCN Regional Uniform Tests on SCN-infested soils (Table 5). In 2005, the yield was 90% of the yield of the third highest yielding public cultivar, 'IAR2001 BSR' (Cianzio et al., 2008) and significantly ( $P = 0.05$ ) higher than the checks, IA2068 (W.R. Fehr, personal communication, ISU, 2005), and IA2052 (W.R. Fehr, personal communication, ISU, 2005). In 2006, the yield was 98% of the highest-yielding cultivar, IA1008, one of the parents of the germplasm line. In 2005, the rank of AR10SDS was seventh in the test, while it was 12th in 2006. In both years, seed yields of AR10SDS were comparable to public cultivars included in the tests.

AR10SDS is classified as early- to mid MG I (Table 4). Averaged over 2003 and 2004, it was 4 d later than Parker and 5 d later than IA1008. In 2003, the maturity was similar to Parker, one of the MG I checks. In 2004, however, AR10SDS was 7 d later than Parker. Maturity data were comparable to other MG I lines in the test in 2005, whereas it was later in maturity compared to MG I checks in 2006 (Table 5). Lodging scores were similar to the public cultivars in the tests, with some variation due to year. The lodging scores varied among years, ranging from 1.8 to 2.3 (Tables 4, and 5), being slightly higher in 2005 than in 2006. In both years, plant height of AR10SDS was similar to the IA1008 parent and comparable to check cultivars (Table 5).

## Botanical Description and Seed Traits

AR10SDS has white flowers, gray pubescence, brown pods, seed with buff hila, yellow seed coat, and dull seed coat luster.

The one hundred seed weight of AR10SDS was similar to IA1008 (Table 6). Seed quality scores were similar among the three genotypes. Protein and oil content of AR10SDS were the highest among the three lines. Fiber and carbohydrate contents were both lower than each of the two parents. These traits could be of interest to the organic farming community.

## Molecular Analysis of Sudden Death Syndrome and Soybean Cyst Nematode Resistance

Of the nine molecular markers associated with SDS resistance, six were polymorphic between the two parents, Satt156, Satt166, Satt226, Satt311, Satt187, and Satt631. Four of the markers were specific to the Ripley parent. Three markers,

Satt188, Satt262, and Satt448, were not polymorphic between the two parents, having similar-size PCR products in all three genotypes, the parents and AR10SDS. Similar results and lack of polymorphism in the same markers were also noticed at the Diers' laboratory during screening for SDS-resistance QTL considered specific to Ripley (B.W. Diers, personal communication, University of Illinois, 2007). The trend was similar in all three individual plants of AR10SDS that were individually analyzed and considered as replications.

The PCR products of four markers, Satt156, Satt166, Satt187, and Satt631, were similar in size between AR10SDS and Ripley, donor of the resistance to SDS (Fig. 1a,b,e,f). The four markers represented only three QTL on LG L, A2, and N. There was no evidence that the resistance QTL on LG O and D2 had been transferred from Ripley to AR10SDS. In contrast, the PCR product sizes of the four markers associated with SDS were different when compared to IA1008, the SDS susceptible parent of the line. The product sizes of Satt226 and Satt311 were similar between AR10SDS and IA1008 and different in Ripley (Fig. 1c,d). The markers Satt156 and Satt166 are linked together representing the same QTL on LG L. The markers Satt187 and Satt631 represented QTLs located on LG A2 and LG N, respectively. The molecular analysis clearly demonstrated that four SDS markers from Ripley were identified in the germplasm line AR10SDS. These results indicate the particular loci associated with SDS resistance in AR10SDS were inherited from Ripley.

Similarly, the three molecular markers, Satt244, Satt275, and Satt547, specific to two SCN resistance QTLs were identified as polymorphic between the parents Ripley and IA1008 (Fig. 2a–c). Markers Satt244 and Satt547 represented the same QTL on LG J, whereas Satt275 represents the QTL on LG G. The PCR products were similar in size between AR10SDS and IA1008 for all three markers, indicating that SCN resistance QTLs in AR10SDS were inherited from the parent IA1008.

## Conclusions

Soybean germplasm line AR10SDS (experimental line AR03-163008, ISURF Docket #03624) is defined as partially resistant to *Fusarium virguliforme*, the fungus that causes SDS in soybean. AR10SDS is one of the first germplasm lines released by the public sector that is partially resistant to SDS. It is the first germplasm line partially resistant to SDS released by Iowa State University. It is also resistant to *Heterodera glycines*, the SCN race 3. It possesses yield and agronomic traits that make AR10SDS comparable to public soybean cultivars of similar maturity. AR10SDS is an  $F_3$ -derived bulk of 60  $F_8$  plants from the cross of Ripley  $\times$  IA1008. The partial resistance of the line to SDS was proven by planting AR10SDS in artificially SDS-infested soil in the field, by screening in the greenhouse, and by applying molecular markers. The molecular analysis confirmed four SDS markers from Ripley were identified in AR10SDS. AR10SDS also has three molecular markers specific to two SCN-resistance QTLs inherited from the SCN resistant parent of the line, IA1008. AR10SDS is of early- mid MG I, 5 d later than Parker and of similar maturity to IA1008. It is adapted to latitudes 40 to 42° N and could be used in breeding programs

desiring to transfer resistance to both soybean diseases, SDS and SCN.

To date, a clear-cut criteria to differentiate among SDS disease score classifications has not been established (Bond, personal communication, SIU, 2010). The classification of AR10SDS as partially resistant was made by the authors on the basis of observations collected during the process of the line development. AR10SDS behaved in some of the SDS screening tests similarly to resistant genotypes identified previously. The SDS symptomatology of AR10SDS over years and locations, however, had not been consistent to deserve the classification of resistant, even though in some years it was superior to genotypes classified as moderately resistant. The fact that not all of the SDS molecular markers possessed by Ripley were transferred to AR10SDS may help explain the variable disease reaction over environments. However, Ripley itself, although classified as resistant, has also shown variable disease ratings over years and locations (B. Diers, personal communication, University of Illinois, 2008). The data to compare AR10SDS and its parent Ripley are not available, as the two genotypes are from different maturity groups and their evaluations are performed in different tests. Once disease criteria are better established for SDS and more is known about the relative importance of the different molecular markers associated with resistance to SDS, it is possible that the classification of AR10SDS as partially resistant may be modified.

## Seed Availability

Seed for research and breeding purposes may be obtained directly from Iowa State University by contacting Silvia R. Cianzio and requesting seed from the ISU Research Foundation (ISURF) Docket #03724. A Material Transfer Agreement (MTA) will be signed between both parties, after which seed will be made available. AR10SDS germplasm line has been deposited with the National Plant Germplasm System and will be available from that source 20 years from the date of publication.

## Acknowledgments

The line was developed with funds provided by the Iowa Soybean Association, the North Central Soybean Research Program, and the United Soybean Board, ISU project number 4403. All funds were assigned to the ISU Soybean Breeding Project for disease and pest resistance. Additional funds were also provided by the Agriculture and Home Economics Experiment Station, Iowa State University, Ames, Iowa 50011-1010. Appreciation is extended to Mr. Troy Cary, Dr. Scott Abney (now retired) and Mr. Wad Crochet, coordinators of the Uniform Regional Tests, for their work, attention to detail, and collaboration in preparing and summarizing data corresponding to the tests. Appreciation is also extended to Ms. Cathy Schmidt, and Dr. J. Bond, coordinators of the SDS Uniform Regional Tests, for their work, attention to detail, and collaboration in preparing and summarizing data corresponding to the tests. Data summarized by Ms. Schmidt and Mr. Cary were used in this registration article with permission, and this is greatly appreciated. Continuous support to conduct SCN Regional Tests is provided to the Illinois Crop and Soil Department, University of Illinois by the United Soybean Board. Appreciation is also extended to both organizations.

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