

Registration of AR11SDS Soybean Germplasm Resistant to Sudden Death Syndrome, Soybean Cyst Nematode, and with Moderate Iron Deficiency Chlorosis Scores

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Abstract

Genetically resistant crops are effective strategies for crop production and the soybean [*Glycine max* (L.) Merr.] program against pathogens at Iowa State University (ISU) breeds germplasm possessing resistance to Iowa pests. Sudden death syndrome (SDS) caused by *Fusarium virguliforme*, soybean cyst nematode (SCN; *Heterodera glycines* Ichinoe), and iron deficiency chlorosis (IDC) are Iowa yield deterrents. AR11SDS (Reg. No. GP-399, PI 675224) was developed at ISU, Project 4403, Agronomy Department, ISU Research Foundation (ISURF) Docket # 3999. AR11SDS is an F₃-derived bulk of 60 F_{8,9} plants from the cross 'Ripley' × 'IA2036'. Ripley resistance to SDS and IA2036 resistance to SCN were crossed in Iowa. Generations were advanced at the ISU research site, University of Puerto Rico, PR. From 2003 to 2006, AR11SDS yield was evaluated. Resistance to SDS and SCN was screened in greenhouses and on SCN-infested fields. Calcareous soils were used to screen IDC. Five SDS resistance quantitative trait loci (QTLs) were from Ripley, and one SCN QTL was from IA2036. Average seed yield on SCN-infested soil (3891 kg ha⁻¹) was similar to the high-yielding SDS-susceptible 'IA2068' (3658 kg ha⁻¹) although lower on non-SCN soil, similar in yield to SDS-susceptible 'IA2065', and superior to SDS-susceptible 'Dwight'. AR11SDS is of early-mid maturity group II, adapted to 40 to 42° N latitude, and is highly resistant to SDS, resistant to SCN (Race 3, HG Type 0, and 7), and moderately resistant to IDC (score 2.4). These traits and its yield and agronomic performances make AR11SDS a unique source for SDS breeding in the northern US soybean region.

PLANTING genetically resistant crops is one of the most effective management strategies for crop production. The use of resistant genotypes does not require additional inputs by agribusiness. It also contributes to maintaining the ecological equilibrium. Other strategies such as the use of cultural practices to improve soil drainage and to reduce soil compaction, as well as crop rotation, are also appropriately used (Roy et al., 1997; Rupe et al., 1997).

Sudden death syndrome (SDS) is an important yield deterrent to soybean [*Glycine max* (L.) Merr.] in most of the major soybean producing states of the United States (Wrather et al., 2010). From 1996 to 2007 in the United States, yield depression by SDS ranked second to fifth compared with all other soybean diseases occurring in the midwestern soybean production region (Roy et al., 1997; Wrather and Koenning, 2009; Wrather et al., 2010). Sudden death syndrome in soybeans was first identified in Arkansas in 1971. The disease spread to other soybean production regions in the United States, and in 1994 it was first identified in southern Iowa (X.B. Yang, personal communication, 1994). Currently, it is observed in all counties of the state of Iowa. Yield losses caused by SDS in Iowa peaked in 2010, due to the unfortunate coincidence of environmental factors such as climate (temperature and rain), soil conditions, and the opportunistic presence of the fungus (Robertson and Leandro, 2010).

The disease caused by the soil-borne fungus *Fusarium virguliforme* (Fv) colonizes soybean roots, producing root rot (Aoki et al., 2003; Rupe, 1989). The pathogen also produces toxins that on translocation to the leaves via the xylem, cause foliar symptoms (Jin et al., 1996; Rupe, 1989). The majority of the resistant cultivars were initially developed for maturity groups (MG) IV and later. Therefore, breeding for resistance to SDS in the northern United States has had to use later-maturing resistant cultivars as donors crossed to high-yielding susceptible cultivars of early-maturity groups (Cianzio et al., 2014).

Soybean cyst nematode (SCN), caused by *Heterodera glycines* Ichinoe, is another major pest in the state of Iowa, as well as throughout the entire soybean production region in the United States (G. Tylka, Iowa State University [ISU], personal

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Abbreviations: DI, disease incidence; DS, disease severity; DX, disease index; IDC, iron deficiency chlorosis; ISU, Iowa State University; ISURF, Iowa State University Research Foundation; LG, linkage group; MG, maturity group; PCR, polymerase chain reaction; QTL, quantitative trait locus; SCN, soybean cyst nematode; SDS, sudden death syndrome; SIU, Southern Illinois University.

communication, 2014). In Iowa, the prevalent nematode race is 3 (HG types 0 and 7), as determined by the ISU Extension Service. Nematodes damage soybean roots and diminish plant height, thereby negatively affecting soybean yields (Wang et al., 2003).

Iron deficiency chlorosis (IDC) in soybean may occur when certain genotypes are planted on calcareous soils (Cianzio, 1999) because genotypes differ in their ability to use soil iron (Fe) under high pH conditions (Weiss, 1943). The high pH values of some soils render the soil Fe present at the Fe^{+3} stage unavailable to the plant due to impaired plant absorption through the roots (Curie and Briat, 2003; Mengel, 1994). Iron deficiency chlorosis affects commercial soybean fields, as soybean production moves north and west in the United States, into soils with high concentrations of carbonate and salts (Coulombe et al., 1984). There are an estimated 1,821,085.40 ha (4.5 million) soybean acres at high risk for iron deficiency problems in the United States alone, distributed mainly in the eastern Dakotas, western Minnesota, and north-central Iowa (N. Hansen, Colorado State University, personal communication, 2007). In Iowa, calcareous soils occur in the northern portion of the state and are classified as Harps soil (fine-loamy, mixed, superactive, mesic Typic Calciaquolls). The IDC symptoms are evident in the interveinal tissue of young leaves, while the veins remain green (de Mooy, 1972). Some genotypes may recover during the growing season; however, yield is reduced whenever yellowing occurs (Niebur and Fehr, 1981).

In Iowa, it is possible to encounter a combination of biotic stress-related problems such as the presence of soybean cyst nematode and *Fusarium virguliforme*. Additionally, abiotic stress factors such as IDC can occur (Charlson et al., 2004). The appearance of multiple yield deterrents in Iowa soils imposes the need to improve germplasm possessing genetic resistances to multiple stress factors. This was the objective of the ISU soybean breeding project for disease, pests, and abiotic resistance in releasing the germplasm line AR11SDS (Reg. No. GP-399, PI 675224). AR11SDS is of early to mid-MG II, adapted to 40 to 42° N latitude. It is highly resistant to SDS, resistant to SCN (Race 3, HG Type 0, and 7), and moderately resistant to IDC (score 2.4). The resistance of AR11SDS to SDS and to SCN, in addition to the moderate resistance to IDC and its yield and agronomic performances make the germplasm AR11SDS a unique source for SDS breeding in the northern US soybean production region.

Methods

Pedigree

AR11SDS (tested as experimental line AR03-263051) is an F_3 -derived bulk of 60 $F_{8,9}$ plants from the cross 'Ripley' × 'IA2036'. Ripley (Cooper et al., 1990) is a high-yielding, lodging resistance determinate cultivar, jointly released by the USDA-ARS and the Ohio Agricultural Research and Development Center in 1985. Ripley, of MG IV and partially resistant to SDS, was derived from the cross 'Hodgson' × V68-1034. Hodgson (Lambert and Kennedy, 1975) is a selection from the cross 'Corsoy' × M372. Corsoy (Weber and Fehr, 1970) was released by Iowa State University. V68-1034 is a selection from the cross 'York' × PI 71506. York (Smith, 1968) was released

by the USDA-ARS. IA2036 (S.R. Cianzio et al., Intellectual Property Disclosure & Record IA2036, ISURF Docket # 02599) is a cultivar released by Iowa State University in 1989 that possesses excellent resistance to SCN, with equal or superior yield to widely grown public cultivars of similar maturity at the time of release. IA2036 was derived from the cross 'Jack' × A86-301024. Jack (Nickell et al., 1990) is resistant to SCN. The genetic source of SCN resistance in Jack is derived from PI 88788. A86-301024 is an Iowa State University advanced experimental line selected from the cross A81-356022 × 'Hack'. Hack was released by the University of Illinois (Nickell et al., 1985).

Line Development and Evaluation of Traits in Iowa

The cross of Ripley × IA2036 was performed in 2001 at the Agronomy and Research Farm at Ames, IA. The cross designated as AX17927 was part of a group of crosses made with the objective to develop high-yielding lines with resistance to SDS. Thirty-seven F_1 seeds were obtained. During the winter of 2000–2001, F_1 and F_2 seed was planted at the ISU research site located at the Isabela Substation, University of Puerto Rico, at Isabela, PR. Morphological markers (flower and pubescence colors) were used to confirm the hybrid nature of the F_1 plants that afterward were harvested in bulk. The F_2 and F_3 generations were advanced at the ISU research site in Puerto Rico. The F_3 seed was harvested, equally sampling each F_2 plant, three seeds per plant. In summer 2001, the F_3 seed was planted at Bruner Farm near Ames, with the objective of harvesting F_3 -derived lines classified by maturity to obtain $F_{3;4}$ lines. During winter 2002, the $F_{3;4}$ lines were screened for SDS resistance/susceptibility under greenhouse conditions at the Cianzio laboratory, Agronomy Department Research Greenhouses, at Ames.

Field test evaluations were conducted in Iowa during 2002 to 2004 using remnant seed of the $F_{3;4}$ seed of the lines evaluated for resistance to SDS during the winter. In 2002, the lines were evaluated in the first yield test at one central location near Ames. The plots were one-row plots, 63 cm long, planted at eight seeds per 30 cm, with two replications. The lines and yield and maturity 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. Each plot was individually harvested, and the superior yielding lines were selected for further testing.

Subsequent field tests were conducted at different Iowa locations in replicated experiments during 2003 and 2004. In 2003, the resistant $F_{3;5}$ lines were evaluated in replicated tests on non-SDS infested soil at two Iowa locations, Kanawha and Crawfordville. In 2004, the experimental line AR03-263051 was evaluated in the third yield test at two locations, Kanawha in a different field than used in 2003, and at Marshalltown, both in IA. At each year-location environment combination, plots of the line and checks were two-row plots, 4.5 m long, spaced between rows by 0.80 m, and planted with 27 seeds m^{-1} . The experimental line AR03-263051 at each location-year combination always had superior yield to existing check cultivars (data not shown).

During each winter from 2002–2003 until 2004–2005, the line was screened for resistance to SDS under greenhouse conditions at the Cianzio laboratory (data not shown). The protocol

used was first developed at Iowa State University by X.B. Yang (Iowa State University, personal communication, 2002), modified by P. Lundeen (Iowa State University, personal communication, 2004), and later patented by D. Lightfoot at Southern Illinois University (SIU) (Lightfoot et al., 2007). The patented screening test was used with permission. At each screening test, the experimental line AR03-263051 showed high levels of SDS resistance (data not shown).

Line Evaluation at Regional Trials

From 2005 to 2006, the experimental line AR03-263051 was evaluated in the Uniform Northern Regional Soybean Cyst Nematode tests, planted in replicated experiments throughout the northern soybean production region (Cary and Diers, 2005, 2006). Four-row plots were planted at several locations in SCN-infested and one noninfested soil. The two center rows were harvested to estimate yield. Experimental lines entered in the tests were also screened for their SDS and SCN resistances under greenhouse conditions and in field tests, as well as for their IDC reaction by plantings on fields with calcareous soil, in Iowa and Minnesota. Since 2007, the line has been used as a check genotype in the SDS Regional Tests, conducted at SIU, Carbondale, IL (J. Bond, SIU, personal communication, 2007).

Agronomic and yield data were collected at each location. In addition, seed composition, SDS, SCN and IDC data were obtained at a smaller number of locations. On the basis of these and data accumulated over the years at the Iowa locations, the decision was made to release the experimental line AR03-263051 as a germplasm line with the identification of AR11SDS.

Screening for Sudden Death Syndrome Resistance under Greenhouse Conditions

Screening tests were conducted at the Cianzio laboratory, Agronomy Department Research Greenhouses at Ames. Three isolates of *F. virguliforme*, “Clinton 1B” from Clinton County and two isolates from Scott County, “ScottF2I11a” or “Scott B2,” were obtained from roots of SDS symptomatic plants from production fields in Clinton and Scott Counties in Iowa. The screening protocol was previously described (Cianzio et al., 2014). The cups were maintained in 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 in the greenhouse were 400 W High Pressure Sodium, model Sylvania 67533-LU400/ECO-HPS.

In the greenhouse, SDS symptoms were evaluated at 35 d after planting at 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 plots 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 for Sudden Death Syndrome Resistance in Field Conditions

The field tests conducted in Iowa were planted in fields where irrigation was available and, when possible, with a history of SDS. Plots were located at the Hinds Farm Experimental Research Station, near Ames (Table 1). A randomized complete block design with three replications was used in each experiment, and the plantings were conducted on plots artificially inoculated with SDS.

The inoculum was prepared at the Cianzio laboratory using white sorghum [*Sorghum bicolor* (L.) Moench] seed inoculated with a mixture of two isolates of *F. virguliforme* obtained from naturally infested fields in Iowa. Five-week-old inoculum was sieved to a maximum size of 0.06 mm (1/4 in) and placed in each of the soybean seed packets 48 h before planting. The amount of 4 cm³ 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 row and in the test were at the R6 reproductive growth stage (Fehr et al., 1971). Disease incidence and disease severity were scored on a plot basis, and the disease index was calculated as $DX = DI \times DS/9$. In all SDS screening tests, susceptible and resistant genotypes were included as control cultivars. The control genotypes were the same as used in the SDS Soybean Variety Tests performed at SIU from 2007 to 2009; ‘SB2859’ and ‘233+RR’ were the SDS-resistant cultivars, and ‘H-2494 RR’ and ‘Beck 299N’ were the susceptible cultivars. In the tests conducted in 2010, the cultivar SB2859 was substituted with ‘LD06-30504RA’.

Soybean Cyst Nematode Screening and Resistance/Susceptibility Assessment

Data reported for SCN screening was collected as part of the disease screenings conducted for all experimental lines planted at the SCN uniform tests (Table 2). Screenings were conducted at Purdue University (West Lafayette, IN), and at the University of Illinois (Urbana-Champaign, IL). The test at Purdue was performed by bringing in SCN-infested soil from field locations and using it to test each soybean line for resistance to the SCN population found in that field. The field populations were classified using the Race system (Schmitt and Shannon, 1992).

The tests conducted at Illinois used sterilized sandy soil artificially inoculated with 1000 eggs (T. Niblack, Ohio State

University, personal communication, 2005). Thirty days after inoculation, female cysts were washed from the roots and

counted. The female index was calculated for each entry by dividing the mean number of cysts on the entry by the mean

Table 1. Average sudden death syndrome (SDS) disease index of AR11SDS and cultivars evaluated in fields in Iowa, Illinois, Minnesota, and Ontario, Canada. Tests were replicated. Plots were of different sizes depending on location.†

| Line and cultivars | Disease index scores by location‡ | | | | |
|--------------------|-----------------------------------|-----------|--------------|------------|----------|
| | 2007§ 14 genotypes evaluated | | | | |
| | Waseca, MN | Paris, IL | Streator, IL | Urbana, IL | Ames, IA |
| Line | | | | | |
| AR11SDS | 4 | 0 | 7 | 7 | 2 |
| Cultivars# | | | | | |
| SB2859 (SDS) | 19 | 13 | 43 | 13 | 18 |
| 233+RR (SDS) | 1 | 0 | 8 | 2 | 1 |
| H-2494 RR | 31 | 21 | 90 | 20 | 22 |
| Beck 299N | 25 | 36 | 71 | 33 | 6 |
| Average | 16 | 8 | 39 | 11 | 7 |
| LSD (0.05) | 22 | 10 | 20 | 11 | 15 |
| | 2008¶ 18 genotypes evaluated | | | | |
| | Havana, IL | Ames, IA | | | |
| Line | | | | | |
| AR11SDS | 1 | 15 | | | |
| Cultivars | | | | | |
| SB2859 (SDS) | 2 | 9 | | | |
| 233+RR (SDS) | 1 | 0 | | | |
| H-2494 RR | 17 | 39 | | | |
| Beck 299N | 9 | 39 | | | |
| Average | 3 | 16 | | | |
| LSD (0.05) | 9 | 13 | | | |
| | 2009¶ 18 genotypes evaluated | | | | |
| | Havana, IL | Ames, IA | | | |
| Line | | | | | |
| AR11SDS | 12 | 4 | | | |
| Cultivars | | | | | |
| SB2859 (SDS) | 19 | 34 | | | |
| 233+RR (SDS) | 5 | 4 | | | |
| H-2494 RR | 29 | 26 | | | |
| Beck 299N | 41 | 30 | | | |
| Average | 17 | 18 | | | |
| LSD (0.05) | 15 | 19 | | | |
| | 2010§ 27 genotypes evaluated | | | | |
| | Harrow, ON | Ames, IA | Kanawha, IA | Urbana, IL | |
| Line | | | | | |
| AR11SDS | 0 | 0 | 0 | 1 | |
| Cultivars | | | | | |
| LD06-30504RA (SDS) | 0 | 4 | 14 | 0 | |
| 233+RR (SDS) | 11 | 2 | 1 | 1 | |
| H-2494 RR | 28 | 15 | 27 | 36 | |
| Beck 299N | 5 | 7 | 23 | 26 | |
| Average | 6 | 3 | 6 | 8 | |
| LSD (0.05) | 15 | 10 | 11 | 12 | |

† The data were extracted from the 2007 through 2010 Northern Regional Sudden Death Syndrome Tests, published by the Southern Illinois University, Agricultural Research Center, Carbondale, Illinois, and used with permission.

‡ Average disease index (DX) of the test = (DI × DS)/9, where DI = disease incidence and DS = disease severity.

§ In 2007, there were 14 entries in the test, including checks; in 2008 and 2009, there were 18 entries in the test, including checks; in 2010 there were 27 entries in the test, including checks.

¶ Kanawha, 2008 and 2009, and Waseca, 2009, averages not included due to low disease indexes of susceptible checks. There was not enough disease pressure to differentiate lines.

SB2859, 233+RR, and LD06-30504RA are SDS-resistant checks; H-2494 RR and Beck 299N are SDS-susceptible checks. "(SDS)" next to cultivar name indicate that the cultivar is resistant to SDS.

Table 3. Average iron deficiency chlorosis (IDC) scores of AR11SDS and cultivars evaluated in fields in Iowa and Minnesota. Tests were replicated.†

| Line and cultivars | IDC | |
|--------------------|------|-----------|
| | Iowa | Minnesota |
| | 1–5‡ | |
| | 2005 | |
| Line | | |
| AR11SDS | 2.4 | 2.3 |
| Cultivars§ | | |
| IA2068 (SCN) | 2.7 | 1.8 |
| IA1008 (I) (SCN) | 3.4 | 2.2 |
| IA2065 | 2.9 | 2.2 |
| Dwight (L) (SCN) | 3.2 | 2.0 |
| | 2006 | |
| Line | | |
| AR11SDS | 1.7 | 3.1 |
| Cultivars | | |
| IA2068 (SCN) | 2.1 | 3.0 |
| IA1021 (I) | 2.6 | 3.1 |
| IA2065 | 2.4 | 3.6 |
| Dwight (L) (SCN) | 2.9 | 3.1 |

† The data were extracted from the 2005 and 2006 Northern Regional Soybean Cyst Nematode Tests, published by the University of Illinois, Dep. of Crop Sciences, Urbana, IL, and used with permission. See Cary and Diers (2005, 2006) for information on screening methods.

‡ Score: 1 = no chlorosis, normal green plants; 2 = slight yellowing of upper leaves and leaf veins with the interveinal area not showing a differentiation in color; 3 = interveinal chlorosis in the upper leaves, with no obvious stunting of growth or death of tissue observed; 4 = chlorosis of the upper leaves observed along with some apparent stunting of growth or necrosis of tissue; 5 = plant death due to IDC. In each state, tests were planted in two locations, each with two replications in Iowa, and three replications in Minnesota. Data reported are averages over locations. A practical interpretation guide is provided in terms of 1 = green leaves; 2 = slight yellowing of upper leaves; 3 = interveinal chlorosis of upper leaves. Also in practical terms, green is preferable to slight yellowing, and slight yellowing preferable to interveinal chlorosis.

§ IA2068, IA1008, and Dwight are SCN resistant; IA1021 and IA2065 are SCN susceptible. IA2068 and IA2065, are moderately tolerant to IDC; IA1008 and Dwight are susceptible to IDC. The IDC ratings are dependent on the environmental conditions.

from each location CV. The estimated variance was then used to calculate Fisher's protected LSD. The LSDs for all traits evaluated in Iowa were calculated using standard ANOVA analysis.

Similar to previous research (Cianzio et al., 2014), a combined LSD across locations and years was not calculated for SDS disease index because of the highly variable disease levels across locations and years. For seed traits, including protein and oil content, estimates were either scored or analyzed from bulking six individual samples per genotype, therefore was no possibility of providing statistical analyses results for any of the traits. Similarly for IDC, scores were communicated as averages of individual replications, therefore there was no possibility of providing statistical results.

Molecular Analysis

Molecular analysis was conducted for the experimental line AR03-263051 to detect the presence of the SDS resistance quantitative trait loci (QTLs) specific to Ripley. A polymerase chain reaction (PCR)-based molecular marker analysis was used. The seeds of AR03-263051, Ripley, and IA2036 were grown in the

greenhouse, and leaf samples were collected for DNA extraction and analysis.

Genomic DNA was isolated according to the hexadecyltrimethylammonium bromide (CTAB) extraction method (CIMMYT, 2005). DNA was isolated separately from leaf samples of five individual plants from each genotype (experimental line and parents), and each of the five samples was treated as five replications. The final DNA pellet was resuspended in 300 µL of 1× TE buffer pH8.0 and stored at –20°C until further use. DNA quality was checked by running 2 µL of the DNA on 1% agarose gel. Also, DNA quantity was estimated by running a 1 Kb DNA ladder (NEB Inc., MA, USA) along with the genomic DNA (not shown). DNA was diluted with sterile water, and 20 ng was used as a template for 10 µL of PCR run. We performed PCR amplification using a thermal cycler program of 2 min at 94°C, 38 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 1 min. A 10-min extension at 72°C followed the last cycle. The PCR reaction mixtures included 2 mM MgCl₂ (Bioline), 0.25 µM each of forward and reverse primer (Integrated DNA Technologies, Inc.), 2 µM deoxyribonucleotides (dNTPs), and 0.5 U Choice Taq DNA Polymerase (Denville Scientific, Inc.).

Five SDS QTLs identified in Ripley as associated with resistance were used in the molecular analysis. The QTLs belong to five different linkage groups (LG): LG L (*Glycine max* (Gm) chromosome 19; LG O (Gm10); LG D2 (Gm17); LG A2 (Gm08); and LG N (Gm03). Specific SDS resistance QTL markers for four of the QTLs were obtained from the published literature (Kazi et al., 2008; Neto et al., 2007). Three markers, Satt156, Satt166, and Satt448, are linked to the QTL located on LG L (qFDS002-03) (Neto et al., 2007). The markers linked to LG D2 QTL (cqSDS001), are Satt311 and Satt226 (Neto et al., 2007). The marker Satt187 is linked to the QTL on LG A2 (qFDS003-06), whereas the marker Satt631 is associated with the QTL on LG N (qSDS002) (Hashmi, 2004; Kazi et al., 2008). Two markers, Satt188 and Satt262, are linked to the LG O QTL (T.I. Pruski, University of Illinois, personal communication, 2011).

The molecular markers for the two SCN-resistance QTL located on LG J (Gm 16) and LG G (Gm 18) specific to the SCN resistant parent IA2036 (SCN resistance donor PI 88788) were designed at the Cianzio laboratory based on the information provided in previous publications (Chang et al., 2011; Glover et al., 2004). Two markers, Satt244 and Satt547 are linked to LG J (Qscn3-3), whereas 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/>).

Polymerase chain reaction was done in MyCycler (BioRad Inc.). The amplified products were resolved on a 4% agarose gel along with either 50-bp or 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 ultraviolet light.

Seed Purification

Seed purification of AR11SDS began at the Agronomy Farm Research Center near Ames in 2007. To obtain breeder seed and seed for increases, 60 individual F_{8,9} plants uniform in maturity,

flower and pubescence colors, and agronomic and seed traits were harvested. During winter 2008, seed derived from the 60 individual plants was checked for hilum color before bulking the F_{8,9} seed for release.

The seed source is maintained by the ISU soybean breeding project for disease resistance, availability for research, and distribution. The line was publicly released in 2012 by the Iowa State University Research Foundation (ISURF) under ISURF Docket # 3999, identified as AR11SDS.

Characteristics

AR11SDS is a germplasm line possessing resistance to SDS and SCN and moderate IDC scores. The seed yield of AR11SDS is similar to public cultivars 'IA2068' and 'IA2065' and is superior to 'Dwight' (Nickell et al., 1998). AR11SDS is of early- to mid-MG II, 2 to 3 d later than IA2068 and IA2065 and of similar maturity to Dwight. AR11SDS is adapted to latitudes from 40 to 42° N and may serve breeding programs for its combination of resistance and yield traits. AR11SDS is a unique germplasm that possesses high resistance to SDS and to SCN and moderate resistance to IDC. Yield and agronomic and maturity traits make the germplasm line a unique source to improve SDS resistance of early maturing soybeans adapted to the northern United States.

Sudden Death Syndrome Resistance

Disease resistance of AR11SDS measured as DX at each of four consecutive years from 2007 to 2010, indicating the line is resistant to SDS (Table 1). Each year, AR11SDS was one of the more resistant lines in the tests. In some years, it was even more resistant than at least one of the SDS resistant checks and comparable in its resistance to the best current resistant check. Every year, except in 2008 at the Ames location, when AR11SDS was planted in the same test as SB2859 (resistant check), the DX index of the ISU line was superior to the control cultivar SB2859. According to LSDs, AR11SDS was not significantly superior to MN1606 in Minnesota in 2007, nor at Illinois in 2008 or 2009. Depending on the year and location, AR11SDS had either superior SDS resistance or slightly less SDS resistance than 233+RR, the highest resistant SDS check in the tests. It is to be noted, however, that both genotypes had variable resistance depending on locations and years, suggesting a complex interaction between the environments and the resistance QTLs of AR11SDS. Overall, AR11SDS resistance to SDS indicates that it is a valuable genotype in the early maturity groups for use as a donor source of SDS resistance.

Soybean Cyst Nematode Resistance

In the tests conducted at Purdue, AR11SDS performed as moderately resistant to Race 3 in 2005 and resistant to Race 3 in 2006 (Table 2). In 2006, AR11SDS also showed resistance to four other races identified on infested soils in Indiana, Races 1, 3, 5, and 6. AR11SDS was, however, moderately resistant, lightly resistant, and moderately susceptible to other race 1 isolates.

In the tests conducted at Illinois, AR11SDS was resistant to populations HG Type 0 in 2005 and HG Type 7 in 2006 (equated in both cases to Race 3 in the race classification system) (Table 2). Under the HG Type system, AR11SDS was

not resistant to HG Type 2.5.7, equated to Race 1 according to the race system. This result was observed in 2005 and 2006. The results support the conclusion that AR11SDS, in addition to being highly resistant to SDS, also possesses resistance to at least one population/race of the nematode, identified as Race 3, HG Type 0, and 7, which is of common occurrence in Iowa (G.L. Tylka, ISU, personal communication, 2014).

Iron Deficiency Chlorosis: Abiotic Factor Resistance Assessment

The IDC scores of AR11SDS were adequate in three of the four environments in which the line was evaluated: the two state locations in 2005 and the Iowa location in 2006 (Table 3). In Iowa 2005 and 2007 and Minnesota 2005, the IDC scores were in the lower half of the 1-to-5 scoring scale. In Minnesota 2006, the IDC score was 3.1, less than desirable. It is important to note, however, that all the lines in that test had similarly low scores. A score of 3.0 or higher from a production standpoint is not acceptable.

On average, then, the results indicate that AR11SDS may possess some favorable genes for resistance to IDC. Depending on environmental conditions at the planting location, the genes could provide protection in soils where iron is not in the available chemical state (Fe⁺²) that is adequate for root absorption by some genotypes. In any case, it is important to consider that IDC symptom expression has been shown to interact with environmental conditions at the locations in which the lines were evaluated (Cianzio, 1999), and on that basis, variation in IDC performance can be expected. Although at first considered as simply inherited (Weiss, 1943), the trait was later reported as being quantitative in nature (Cianzio and Fehr, 1980, 1982), which may help explain the interaction between the genetic constitution of the line and the environment in which the lines are evaluated.

Yield and Agronomic Performance

AR11SDS had average yield performance within or close to the superior range of all the lines included in the 2005 and 2006 tests that were evaluated in the SCN-infested and non-infested soils (Table 4). The 2-yr summary shows AR11SDS yielded 3891 kg ha⁻¹ on SCN-infested soil, while the two best high-yielding cultivars of the tests yielded 3884 kg ha⁻¹ for IA2068 and 3941 kg ha⁻¹ for Dwight. Under noninfested soils, AR11SDS yielded 4341 kg ha⁻¹, less than IA2068 (4597 kg ha⁻¹), similar to IA2065 (4341 kg ha⁻¹), and better than Dwight (3695 kg ha⁻¹). IA2068 and Dwight are SCN-resistant cultivars, while IA2065 is susceptible to SCN. The three cultivars are SDS-susceptible. The different yields under both SCN-infested and noninfested soils between the germplasm line AR11SDS and the control cultivars IA2068, IA2065, and Dwight were not significantly different according to the LSDs in each test.

In individual years and on noninfested soil, the yield observed for AR11SDS in 2005 was less than in 2006 (Table 4). AR11SDS yielded better in 2006 (5037 kg ha⁻¹) than in 2005 (3645 kg ha⁻¹). Although the yield observed in 2005 for AR11SDS was less than in 2006, AR11SDS in 2005 still yielded similar to the average of the checks; both yields were 3645 kg ha⁻¹. Under non-infested conditions in 2005, AR11SDS yield was 3645 kg ha⁻¹,

superior to the yield of IA1008 (3470 kg ha⁻¹) and of Dwight (3329 kg ha⁻¹). Also in 2005, AR11SDS yielded less than IA2068 and IA2065. In 2006 under noninfested conditions, AR11SDS had superior yields to IA1021, IA2065, and Dwight, although less than IA2068. These observations need to be considered with caution, however, because they were the result of only one test location each year.

On infested soil, yield ranking of the AR11SDS line ranged from 6 among 28 genotypes in 2005 to 13 among 24 genotypes

in 2006 (Table 4). There were 10 sites in 2005 and 13 in 2006. In 2005, AR11SDS ranked 9 under infested conditions and yielded better than the average of the checks in the tests, and even superior to SCN-resistant cultivars IA2068 and IA1008, which ranked 11 and 20, respectively. In 2006, AR11SDS ranked 13, IA2068 ranked 8, and IA1021 and IA2065 ranked 23 and 21, respectively. In noninfested soil in both 2005 and 2006, the ranks only pertain to one site. AR11SDS occupied rank 9 in 2005 and 6 in 2006. In 2005, IA2068 was ranked

Table 4. Average seed yield and agronomic performance of AR11SDS and cultivars evaluated in the northern region of the United States, 2005–2006. Tests were replicated and planted on soybean cyst nematode-infested and noninfested soils. Plots were multiple rows of different sizes depending on location, with the middle two rows harvested for yield.†

| Line and cultivars | Yield | | | | Maturity date 1 Sept. = Day 1 | Lodging score 1–5‡ | Plant height cm |
|-------------------------------------|---------------------------------|------|------------------------------------|------|----------------------------------|-----------------------|--------------------|
| | Infested kg ha ⁻¹ | Rank | Noninfested kg ha ⁻¹ | Rank | | | |
| 2005§ 28 genotypes evaluated | | | | | | | |
| No. of tests | 10 | | 1 | | 11 | 12 | 10 |
| Line | | | | | | | |
| AR11SDS | 4197 | 6 | 3645 | 9 | 24 Sept. | 1.8 | 94 |
| Cultivars¶ | | | | | | | |
| IA2068 (SCN) | 4109 | 11 | 3874 | 5 | 19 Sept. | 2.0 | 81 |
| IA1008 (I) (SCN) | 3732 | 26 | 3470 | 15 | 17 Sept. | 1.6 | 89 |
| IA2065 | 3907 | 20 | 3907 | 3 | 20 Sept. | 1.5 | 79 |
| Dwight (L) (SCN) | 4317 | 2 | 3329 | 18 | 24 Sept. | 1.9 | 86 |
| Average | 4045 | | 3423 | | | | |
| Average of checks | 4008 | | 3645 | | | | |
| SE of difference | 175 | | 188 | | | | |
| CV (means difference) | 0.0342 | | 0.0559 | | | | |
| 2006§ 24 genotypes evaluated | | | | | | | |
| No. of tests | 13 | | 1 | | 10 | 14 | 11 |
| Line | | | | | | | |
| AR11SDS | 3584 | 13 | 5037 | 6 | 25 Sept. | 1.6 | 99 |
| Cultivars¶ | | | | | | | |
| IA2068 (SCN) | 3658 | 8 | 5319 | 2 | 21 Sept. | 1.4 | 81 |
| IA1021 (I) | 2999 | 23 | 4721 | 13 | 13 Sept. | 1.5 | 76 |
| IA2065 | 3147 | 22 | 4775 | 12 | 22 Sept. | 1.3 | 76 |
| Dwight (L) (SCN) | 3564 | 14 | 4042 | 23 | 25 Sept. | 1.3 | 84 |
| Average | 3484 | | 4741 | | | | |
| Average of checks | 3342 | | 4714 | | | | |
| SE of difference | 242 | | 323 | | | | |
| CV (means difference) | 0.0336 | | 0.1484 | | | | |
| 2005–2006- 2-yr summary | | | | | | | |
| No. of tests | 23 | | 2 | | 21 | 26 | 21 |
| Line | | | | | | | |
| AR11SDS | 3891 | 7 | 4341 | 4 | 24 Sept. | 1.9 | 97 |
| Cultivars¶ | | | | | | | |
| IA2068 (SCN) | 3884 | 8 | 4597 | 2 | 20 Sept. | 1.7 | 81 |
| IA2065 | 3527 | 9 | 4341 | 4 | 21 Sept. | 1.4 | 78 |
| Dwight (L) (SCN) | 3941 | 8 | 3685 | 9 | 25 Sept. | 1.6 | 85 |
| Average | 3762 | | 4082 | | | | |
| Average of checks | 3638 | | 4180 | | | | |
| SE of difference | 40 | | 135 | | | | |
| CV (means difference) | 0.0109 | | 0.0328 | | | | |

† The data were extracted from the 2005 and 2006 SCN UTII Northern Regional Soybean Cyst Nematode Uniform Tests, published by the University of Illinois, Dep. of Crop Sciences, Urbana, IL, and used with permission.

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

§ In 2005, 28 entries were planted in the test, including checks; in 2006, there were a total of 24 entries in the test including checks.

¶ IA2068, Dwight, and IA1008 are SCN resistant; IA1021 and IA2065 are SCN susceptible.

5, IA1008 was ranked 15, and IA2065 was ranked 3. In 2006, IA2068 was 2, IA1021 was 13, and IA2065 was 12.

In terms of maturity and averaged over the 2 yr, 2005 and 2006, AR11SDS was 4 d later than IA2068, 3 d later than IA2065, and 1 d earlier than Dwight. In each individual year, AR11SDS ranged in maturity from 5 d later than IA2068 in 2005 to 4 d later in 2006. AR11SDS was 4 d later than IA2065 in 2005 and 3 d later in 2006. In both years, Dwight and AR11SDS had the same maturity date. Lodging scores and plant height of AR11SDS were comparable to other genotypes in the tests, with AR11SDS showing a tendency to be taller than the checks.

Botanical Description and Seed Traits

AR11SDS is of early to mid-MG II. It has purple flowers, gray pubescence, brown pods, and seeds with buff hila, yellow seed coat, and dull seed coat luster.

Seed traits, such as seed quality scores and seed size of AR11SDS averaged over 2005 and 2006 were comparable to public cultivars (Table 5). The protein content of AR11SDS was higher than the checks. Under the environmental conditions in which the line was evaluated, there seemed to be an advantage that favors the protein content of AR11SDS. Oil content was slightly less than two of the checks and comparable to Dwight.

Molecular Analysis of Sudden Death Syndrome and Soybean Cyst Nematode Resistance

Six molecular markers were associated with SDS resistance in Ripley for which the two parents were polymorphic; Satt166, Satt187, Satt226, Satt262, Satt311, and Satt631 (Fig. 1a–1f). The markers Satt311 and Satt226 are linked together and represent the same QTL on LG D2. The PCR products of each of the six markers were similar in size between the AR11SDS germplasm line and the Ripley parent (Fig. 1a–1f). In contrast, the PCR product size for the mentioned six markers was different when compared to the other parent (IA2036), the SDS-susceptible cultivar. Trends were similar in each of the three plants from AR11SDS that were individually analyzed and considered as replications. Two markers, Satt188 and Satt448, despite

being associated with SDS resistance in the Ripley parent, were not polymorphic between the two parents of AR11SDS germplasm line. Similar results and the lack of polymorphism in the same markers was also noticed at the Diers' laboratory while screening for the SDS resistance QTL considered specific to Ripley (B.W. Diers, University of Illinois, personal communication, 2007). Our molecular analysis clearly demonstrated that the five SDS resistance QTLs from Ripley were transferred to its progeny, AR11SDS.

Similarly, three molecular markers specific to two SCN resistance QTLs were tested. Two markers, Satt275 and Satt547, representing the QTL at LGs G and J, respectively, were polymorphic between the two parents of the germplasm line (Fig. 2). The marker Satt244 was not polymorphic between the parents; therefore, it was not used in the analysis. Of the two polymorphic markers, only Satt275 PCR products were similar in size between AR11SDS and IA2036 (Fig. 2), which indicates that the resistant QTL at LG G in AR11SDS was inherited from IA2036. There was no evidence that the resistance QTL on LG J was transferred from IA2036 to AR11SDS.

Discussion

AR11SDS soybean germplasm is the second SDS-resistant germplasm line developed and released by Iowa State University. AR11SDS is a half-sib of AR10SDS (Cianzio et al., 2014), which was released and registered in 2014. They both share Ripley as the SDS-resistant donor parent. However, the SDS resistance of AR11SDS is superior to that of AR10SDS.

Although research has not been conducted yet to directly compare the two germplasm lines, observations indicate that AR11SDS appears to have superior SDS resistance than AR10SDS. A possible explanation of the differences in SDS resistance between the two germplasms could relate to the fact that AR11SDS inherited two additional QTLs from Ripley than did AR10SDS (Cianzio et al., 2014). AR10SDS inherited three QTLs, one representing each of LG L, A2, and N, whereas AR11SDS inherited five QTLs and from Ripley, representing LGs L (Satt166 marker), A2 (Satt187 marker), D2 (Satt226 and Satt311 markers), O (Satt262 marker), and N (Satt631 marker).

Table 5. Average seed size (100 seed weight), seed quality, and seed composition of AR11SDS and cultivars. Moisture content of the seed was determined, and seed composition was adjusted to a 13% moisture basis.†

| Line and cultivars | Seed (13% moisture) | | | |
|--------------------|-------------------------|--------------------------|--------------------|--------------------|
| | Quality score‡ | Weight | Protein | Oil |
| | 1–5 | g 100 seed ⁻¹ | g kg ⁻¹ | g kg ⁻¹ |
| | 2005–2006 2-yr averages | | | |
| No. of tests | 19 | 23 | 21 | 21 |
| Line | | | | |
| AR11SDS | 2.3 | 13.4 | 405 | 195 |
| Cultivars§ | | | | |
| IA2068 (SCN) | 2.5 | 13.7 | 367 | 208 |
| IA2065 | 2.0 | 14.9 | 389 | 223 |
| Dwight (L) (SCN) | 2.2 | 14.1 | 396 | 194 |

† The data were extracted from the 2005 and 2006 Soybean Cyst Nematode (SCN) UTII and averaged over the two years, from the Northern Regional Soybean Cyst Nematode Uniform Tests, published by the University of Illinois, Dep. of Crop Sciences, Urbana, IL, and is used with permission.

‡ Seed quality score considers amount and degree of wrinkling, defective seed coat, greenishness, and moldy or rotten seed: 1 = very good, 2 = good, 3 = fair, 4 = poor, 5 = very poor.

§ IA2068 and Dwight are resistant to SCN; IA2065 is SCN sensitive.

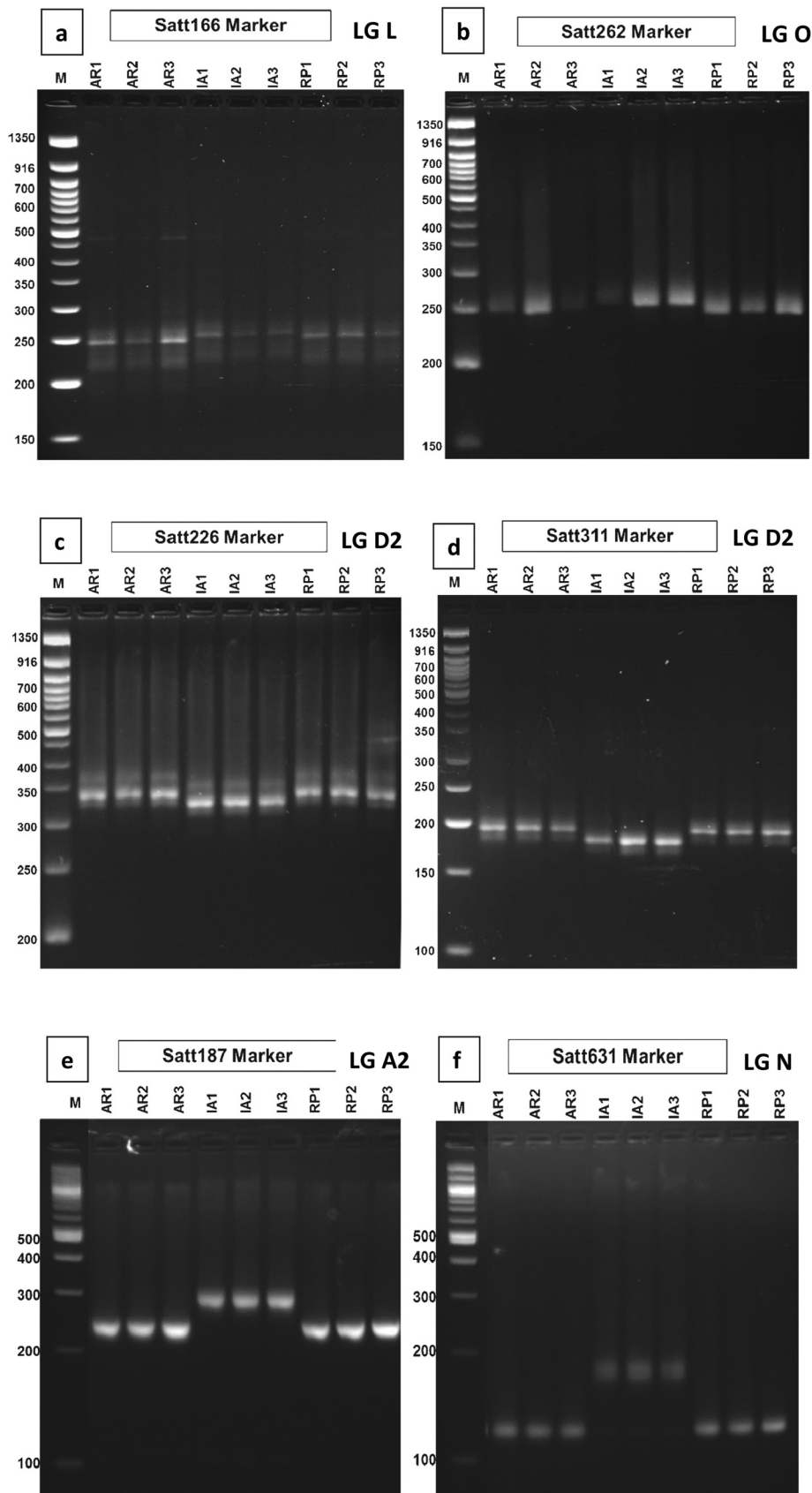


Fig. 1. Agarose gel photographs of polymerase chain reaction (PCR) products of AR11SDS (AR1, AR2, AR3), IA2036 (IA1, IA2, IA3), and Ripley (RP1, RP2, RP3) with different molecular markers. Three replications were included for each genotype. Either a 50- or 100-bp DNA ladder (M) from New England Biolabs was loaded into each gel to show the size of the PCR products. Markers (a) Satt166, (b) Satt262, (c) Satt226, (d) Satt311, (e) Satt187, and (f) Satt631 are associated with sudden death syndrome resistance and were polymorphic among parents of AR11SDS. All resistance loci were inherited from Ripley. The linkage group (LG) of each marker is shown on the top of each figure.

Between AR11SDS and Ripley, there are also six markers in common.

Linkage group D2 and LG O are additional linkage groups present in AR11SDS that were not observed in AR10SDS.

This observation suggests that different linkage groups associated with SDS resistance may contribute differently to the final expression of the SDS resistance trait.

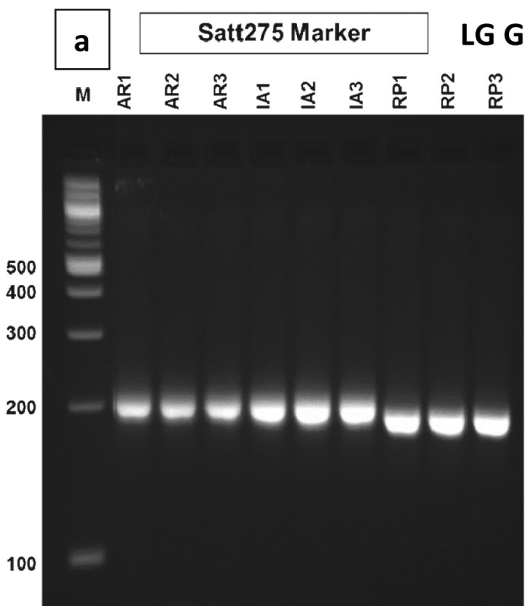


Fig. 2. Agarose gel photograph of polymerase chain reaction (PCR) products of AR11SDS (AR1, AR2, AR3), IA2036 (IA1, IA2, IA3), and Ripley (RP1, RP2, RP3) with different molecular markers. Three replications were included for each genotype. A 100-bp DNA ladder (M) from New England Biolabs was loaded in each gel to show the size of the PCR products. Marker (a) Satt275 is associated with soybean cyst nematode resistance and was polymorphic among the parents of AR11SDS. The resistance locus was inherited from IA2036.

Caution should be taken, however, in this discussion since the genetic background of the susceptible parent is different in each of the two germplasm lines. To establish a true comparison on the relative importance of the different linkage groups associated with SDS resistance, it would first be necessary to individually introgress each of the linkage groups and associated QTLs into the same genetic backgrounds, such as in the development of near-isogenic lines, where each isoline would differ only in the specific QTL that was introgressed. Only then can these observations be tested and pure comparisons made and interpreted.

Seed Availability

Seed of AR11SDS has been deposited with the National Plant Germplasm System and will be available from that source 20 yr from the date of publication. Seed for research and breeding purposes may be obtained directly from Iowa State University by contacting Silvia R. Cianzio, and requesting ISURF Docket # 3999. A Material Transfer Agreement (MTA) will be signed between both parties after which seed will be made available.

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