

Registration of 'IAR1902 SCN' Cultivar Resistant to Soybean Cyst Nematode and Brown Stem Rot

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Abstract

One of the main objectives of the Iowa State University (ISU) soybean [*Glycine max* (L.) Merr.] breeding program is to develop high-yielding cultivars with defensive traits. 'IAR1902 SCN' (Reg. no. CV-533, PI 690449) is a high-yielding cultivar that traces soybean cyst nematode (SCN) resistance to 'Peking'. IAR1902 SCN was developed by ISU Project No. 4403 (Agronomy Department) and released by Iowa State University Research Foundation (ISURF) Docket # 04332. The parental lines of IAR1902 SCN are Agripro 97284-N00-47977 × 'IAR2001 BSR'. The material and transfer agreement corresponding to the Agripro parent indicated that the experimental line has the Peking-type SCN resistance. The IAR2001 BSR parent traces brown stem rot resistance to the cultivar BSR101. Crossing and line development was accomplished in 2005 in Puerto Rico. Field evaluations for yield were performed from 2008 to 2012. Screenings for SCN resistance were performed from 2006 to 2012 in both greenhouse and field conditions. IAR1902 SCN belongs to maturity group (MG) I similar to IA1022 and has yields superior to the current SCN-resistant public cultivars evaluated in the tests. IAR1902 SCN is highly resistant to SCN *Heterodera glycines* (HG) type 0 and resistant to HG type 2.5.7., adapted to 40° to 42° N latitude. Cianzio's laboratory confirmed that IAR1902 SCN has the full-length sequence repeat of three copies of the major Peking-type *rhg1* gene and Peking-type *Rhg4* gene. IAR1902 SCN is also resistant to brown stem rot (BSR). Molecular analyses confirmed that IAR1902 SCN inherited two major BSR resistance quantitative trait loci, *Rbs1* and *Rbs3*, from IAR2001 BSR. IAR1902 SCN has acceptable resistance to iron deficiency chlorosis (IDC) and sudden death syndrome (SDS). IAR1902 SCN will grow well in the northern soybean production region of the United States in which SCN is prevalent and other biotic and abiotic stress factors such as BSR, SDS, and/or IDC may be present.

SOYBEAN [*Glycine max* (L.) Merr.] is affected by both biotic and abiotic stress factors that ultimately reduce seed yield. The soybean cyst nematode (SCN) [*Heterodera glycines* (Ichinohe)] is one of the most important yield deterrents (Bradley and Allen, 2014), widely distributed in the US soybean production region and in southern Canada (Tylka and Marrett, 2014). A soil survey in Iowa indicated that the nematode was present in 99% of the counties of the state (www.plantpath.iastate.edu/scn/). The major concern is that once the nematode is in the soil, it will remain there for a long time (G. Tylka, personal communication, 2000).

The nematode attacks soybean roots, causing disruption to the plant nutrient absorption and to the biology of the soybean plant, by directly feeding on the plant vascular tissue (Riggs and Wrather, 1992). The nematode eggs within the cysts survive in the soil for long time. Upon hatching, cysts release the eggs from which the second-stage juveniles (J2) develop. These juveniles are attracted to soybean roots, and the damaging process to the plant begins. Crop management options to control nematodes in the soil are mainly limited to planting of resistant cultivars and rotation with non-host crops (Concibido et al., 2004). Planting resistant cultivars is the primary and most efficient method to control SCN (Arelli et al., 2015).

Host plant resistance to SCN is a complex scenario, since SCN in the soil is composed of numerous individuals that are genetically heterozygotes, making the population highly heterogeneous in its genetic makeup (Niblack et al., 2002; Riggs and Wrather, 1992). Sexual reproduction of the nematode occurs in the soil, which results in genetic recombination. This plus the selection pressure exerted by resistant genes in the soybean host may trigger the appearance of new *Heterodera glycines* (HG) types (Arelli et al., 2015; Niblack et al., 2008). An additional concern is the narrow genetic base of SCN resistance available

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Journal of Plant Registrations
doi:10.3198/jpr2018.11.0077crc

Received 30 Nov. 2018.

Accepted 16 Apr. 2019.

Registration by CSSA.

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Abbreviations: BSR, brown stem rot; FI, female index; HG, *Heterodera glycines*; IDC, iron deficiency chlorosis; ISU, Iowa State University; ISURF, Iowa State University Research Foundation; MG, maturity group; PCR, polymerase chain reaction; qPCR, quantitative polymerase chain reaction; QTL, quantitative trait loci; SCN, soybean cyst nematode; SDS, sudden death syndrome; SSR, simple sequence repeat.

in commercial soybean cultivars (Arelli et al., 2015; Concibido et al., 2004). It has been reported that 95% of the SCN-resistant commercial cultivars planted in the US Midwest trace to PI 88788, while other sources, such as 'Peking', PI 437564, PI 89772, PI 90763, and PI 209332 have been used less frequently (Tylka and Mullaney, 2015).

To identify the SCN populations in different locations and facilitate SCN resistance breeding, the original race classification has been substituted by the definition of HG type, on the basis of which soybean differential is overcome by the specific SCN population present (Niblack et al., 2002). The HG type concept is therefore the designation used throughout this manuscript.

From a genetic point of view, independent SCN-resistant genes had been identified (Caldwell et al., 1960; Thomas et al., 1975). Rao-Arelli et al. (1992) identified the gene *rhg1*. An additional dominant SCN-resistant gene, *Rhg*, was identified in the cultivar Peking by Myers and Anand (1991), and it is linked to the recessive allele *i* that codes for black seed coat (Caviness, 1992). Quantitative trait loci (QTL) consistently associated with SCN resistance have also been identified on chromosomes (Chr) 18 (Kim et al., 2010) and 8 (Concibido et al., 1997; Concibido et al., 2004). Copy numbers of the *rhg1* gene play an important role in SCN resistance expression (Cook et al., 2012). PI 88788 possesses nine copies of *rhg1* (*rhg1-b* allele), and the gene itself displays incomplete dominance (Concibido et al., 1997; Cook et al., 2012). Resistance to SCN in Peking requires the *Rhg4* gene in addition to three copies of *rhg1* (*rhg1-a* allele) (Liu et al., 2012).

Brown stem rot (BSR) of soybean is caused by the soil-borne fungus *Phialophora gregata* (Allington & D. W. Chamb.) W. Gams. The disease BSR is another soybean yield deterrent that occurs mainly in the northern soybean production region in the United States and southern Canada (Harrington and McNew, 2003; Koenning and Wrather, 2010; Malvick and Grunden, 2008). The management of BSR is a combination of practices that includes tilling and rotation with non-host crops (Tabor et al., 2003). As with SCN, planting of BSR-resistant cultivars is one of the most effective means to reduce yield losses (Bachman et al., 1997).

The objective of the Iowa State University (ISU) soybean breeding program for yield and defensive traits is to release high-yielding soybean cultivars that contribute to expand the genetic base of the soybean commodity, mostly in maturity groups (MG) I to III. 'IAR1902 SCN' (Reg. no. CV-533, PI 690449) is a unique cultivar that traces resistance to SCN from the Peking source. IAR1902 SCN was developed by ISU Project No. 4403 (Agronomy Department) and released by the ISU Research Foundation (ISURF) Docket # 04340. IAR1902 SCN is a bulk of 60 F₃ plants uniform in plant and seed traits with the parentage Agripro 97284-N00-47977 × 'IAR2001 BSR', identified as cross AX20225. The Agripro line is resistance to SCN, and its resistance traces to Peking. The parent IAR2001 BSR (ISURF Docket # 03542; Cianzio et al., 2008) is resistant to BSR, and its resistance traces to PI 84946-2 (Chamberlain and Bernard, 1968). IAR1902 SCN is of MG I (mid to late), similar in maturity to IA1022, and matures about 8 d later than 'Sheyenne' (relative maturity 0.8; Helms et al., 2008;). Yield of IAR1902 SCN is superior to SCN-resistant public cultivars that were

evaluated simultaneously. IAR1902 SCN is adapted to soybean fields in the northern soybean production region, 40° to 42° N latitude, and is highly resistant to SCN HG type 0 and either highly resistant or resistant to HG type 2.5.7. It is also resistant to BSR, with disease scores similar to the IAR2001 BSR parent. IAR1902 SCN has an iron deficiency chlorosis (IDC) score comparable to other public cultivars included in the same tests. It also has an acceptable disease resistance score for soybean sudden death syndrome (SDS; caused by *Fusarium virguliforme* O'Donnell & T. Aoki). IAR1902 SCN will serve in production conditions in which SCN is prevalent, BSR may also be a threat, and SDS and the abiotic stress factor IDC might be present.

Methods

Pedigree

IAR1902 SCN (tested as experimental line AR09-191018) is an F₉ plant selection from the cross Agripro 97284-N00-47977 × 'IAR2001 BSR'. The parent IAR2001 BSR is a product of the cross Pioneer 9233 × 'IA2050' (Cianzio et al., ISURF Docket # 02674). IAR2001 BSR is a high-yield brown stem rot (BSR) resistant cultivar that traces BSR resistance to 'BSR 101' (Tachibana et al., 1987), which in turn traces BSR resistance to the donor source PI 84946-2. Pioneer 9233 is a high-yielding line from Pioneer Hi-Bred International (now Corteva Agriscience), crossed with permission. Agripro 97284-N00-47977 is an experimental line developed by Agripro Seeds Inc. (later owned by Syngenta Seed Co, now owned by Chinachem Group), crossed with permission. Agripro 97284-N00-47977 derives its SCN resistance from the cultivar Peking, as per written company records, and it was made available to Cianzio as part of the crossing agreement.

Line Development

The cross Agripro 97284-N00-47977 × IAR2001 BSR was made at the ISU research site located at the Isabela Substation-EEA, University of Puerto Rico, Isabela, PR, in May to July 2005. It was designated as AX20225 and was part of a group of crosses with the objective of developing high-yielding lines with resistance to SCN. Seven F₁ seeds were obtained. In August 2005, F₁ seed was planted in Puerto Rico and F₁ plants were individually harvested and identified. The hybrid nature of the F₁ plants was confirmed by the segregation of morphological traits. In November 2005, the F₂ seed of AX20225 was planted at Isabela, maintaining identity of the F₁ plant. Each F₂ plant was equally sampled by harvesting three F₃ seeds per plant that were bulked. In February 2006, a similar procedure was followed to harvest the F₄ seed that was sent to Ames, IA. Maturity classification was conducted in 2007 by planting the F₄ seed of AX20225 at the Bruner Farm, near Ames. A total of 485 F₄ individual plants (F₅ seed) were harvested for the three maturity groups adapted to Iowa, MG I, II, and III. During fall–winter 2007–2008, 10 individual F₅ seeds of each F₄ plant classified by maturity during 2007 were screened for SCN resistance under greenhouse conditions (screening protocol described in the following section). A total of 53 individual F_{4,5} plants were selected as SCN resistant (11 of early, 40 of mid, and 2 of late maturities). F₄ remnant seed of each resistant plants was used to continue the line development process.

Soybean Cyst Nematode Resistance Screening

Greenhouse Conditions

Screening tests were conducted at the Cianzio laboratory (data not shown), at ISU in 2008 and 2009 using the SCN screening protocol developed by G. Tylka (personal communication, 2000). Soil infested with SCN, HG type 0, collected from Muscatine, IA, and brought to the greenhouse, was used in screening. Two F₅ seeds of each individual F₄ plant of the cross AX20225 were planted in cone-tainers. The cone-tainers were placed in buckets in a water bath maintained at 27°C ± 1°C to ensure constant soil temperature favoring cyst development. A 14-h photoperiod was simulated using 400 W high pressure sodium light bulbs, model Sylvania 67533-LU400/ECO-HPS. The plants (genotype or entry), in cone-tainers were arranged in the greenhouse following a randomized complete block design with three replications. Plants were watered once daily to maintain soil moisture. Five days after plant emergence, the seedlings were thinned so that only one plant remained in each cone-tainer.

Approximately 30 d after planting, individual plants were pulled and roots were washed with distilled water to dislodge white female cysts (full of eggs). Cyst numbers were counted under a stereomicroscope, and female index (FI) was calculated for each genotype as follows:

$$\text{FI} = [\text{number of cysts in genotype/number of cysts in susceptible cultivar Lee 74 (Caviness et al., 1975)}] \times 100.$$

The genotypes (entries) were rated as highly resistant if the FI was less than 10%; resistant if the FI was from 10 to 24%; moderately resistant if the FI was from 25 to 39%; low resistant if the FI was from 40 to 59%; and as not possessing effective resistance if the FI was more than 60%. The resistant genotypes were evaluated in the first year yield tests for seed yield and agronomic traits at Ames, Iowa in 2008.

In 2013 during the seed purification process two plants in the F₈ generation from each of 60 progeny rows of IAR1902 SCN, uniform in agronomic traits were harvested individually to test SCN resistance under greenhouse conditions, using the protocol previously described. On this screening, two replications were planted for each genotype. Four weeks following planting, roots were harvested, washed, and cysts counted. An analysis of variance was conducted (SAS Institute, 2000) using the average of the two replications of each genotype.

Soybean Cyst Nematode Northern Uniform Tests

Data for SCN resistance were also obtained from nematode screenings conducted as part of the SCN Uniform Tests, Northern Region from 2010 to 2012 (Table 1). Screenings were performed at the University of Illinois following the protocol described in the Northern Regional Soybean Cyst Nematode Tests (<https://cropsciences.illinois.edu/research/scn-tests>). The SCN screening tests were conducted using a randomized complete block design with three replications. The SCN populations were identified as HG type 0 and HG type 2.5.7. (Niblack et al., 2002).

Seed of each genotype was placed on germination paper in an incubator at 27°C ± 1°C for 3 d. One healthy seedling from

each entry was planted in individual cone-tainers filled with sterilized sandy soil and inoculated with 1000 SCN eggs. In the greenhouse, cone-tainers with seedlings were placed in a water bath at a constant temperature of 27°C ± 1°C. Thirty days after planting, plants were uprooted and female cysts were washed from the roots and counted. The FI was calculated, and each genotype was classified as previously described on the basis of the number of cysts counted.

Brown Stem Rot Screening

Brown stem rot resistance screenings were performed in the Cianzio laboratory during August 2013 following the protocol described by McCabe et al. (2016) and Tabor et al. (2003). Seed of IAR1902 SCN (as experimental line AR09-191018) was germinated on germination paper, rolled, and placed in a clear plastic container with 1.5 cm of water. The container was sealed with a plastic lid and stored at 19°C under 16 h of fluorescent and incandescent light (light bulb specifications as described for SCN screening). A week after planting, one seedling was transferred to each cone-tainer, and cone-tainers were placed in a growth chamber following a randomized complete block design with three replications. Temperature in the growth chamber was set at 19°C ± 1°C. When plants were 2 wk old, they were artificially inoculated with the *Oh*₂₋₃ *Phialophora gregata* isolate, which is the isolate most commonly used for all BSR-related work.

Foliar severity was assessed 5 wk after inoculation at the V₄ plant stage (Fehr et al., 1971). The following visual scale was used: 1 = plant death, 2 = plant stem is green and has no leaves, 3 = plant predominantly chlorotic with necrotic leaves, 4 = some plant stunting with mosaic chlorosis and necrosis on leaves, 5 = plant with normal leaf area with some leaves showing yellowing, 6 = plant appears small but healthy, and 7 = plant appears healthy and normal in height. IAR1902 SCN was represented by 60 individual plants along with check cultivars. The average score of each genotype was calculated and analyzed (SAS Institute, 2000).

Sudden Death Syndrome Screening

The SDS screening for genotypes in the Northern Regional Soybean Cyst Nematode Tests was conducted at the Southern Illinois University in Carbondale, IL (Table 1). A randomized complete block design with three replications was used and the plantings were conducted on plots artificially inoculated with the *Fusarium virguliforme*, the SDS-causing fungus (J. Bond, personal communication, 2005). All disease scores were interpolated to the R6.2 soybean growth stage. Disease incidence and disease severity were scored for each genotypes on a plot basis, and the disease index was calculated as (disease incidence × disease severity)/9, where disease incidence represents the percentage of plants in the plot with visible symptoms. The SDS disease severity score was calculated as the average percentage of total foliar surface lost to necrosis or chlorosis on plants with SDS symptoms on a plot basis, using a scale of 1 to 9 for evaluation (1 = 1–10% of the leaf surface chlorotic or 1–5% necrotic; 2 = 11–20% of the leaf surface chlorotic or 6–10% necrotic; 3 = 21–40% of the leaf surface chlorotic or 11–20% necrotic; 4 = 41–60% of the leaf surface chlorotic or

21–40% necrotic; 5 = >60% of the leaf surface chlorotic or >40% necrotic; 6 = <1/3 premature defoliation; 7 = 1/3 to 2/3 premature defoliation; 8 = >2/3 premature defoliation; and 9 = plant death before normal defoliation due to senescence).

Yield Tests and Seed Traits

Field tests for yield evaluations began in 2008. The SCN-resistant F_{4,5}-derived lines were planted to determine yield and agronomic traits for the first time in Iowa, and one replication of single-row plots was grown at each of two locations. From the test, four F_{4,5}-derived lines were selected on the basis of yield and agronomic traits. During winter 2008–2009, the SCN resistance of the selected lines was evaluated under greenhouse conditions at the Cianzio laboratory to confirm previous resistance results. The SCN screening protocol is described under section “Soybean Cyst Nematode Resistance Screening” above.

In summer 2009, the selected F_{4,6} lines of AX20225 were grown in two replications of two-row plots at each of two

locations, Mason City and Arlington, IA. Arlington had an initial SCN population with an egg count of 560 eggs 100 cm⁻³ of soil belonged to HG type 2.5.7. In Mason City, the SCN initial population had an egg count of 600 eggs 100 cm⁻¹ of soil belonged to HG type 7. The highest-yielding F_{4,7}-derived line of AX20225 was evaluated in the 2010 SCN Uniform Soybean Tests Test in 10 different locations (Table 2). In 2011, 12 locations were planted, and 11 locations were planted in 2012. At each location, two replications were planted in either three- or four-row plots, depending on the location and collaborator. From 2010 (F_{4,7}-derived line), to 2012 (F_{4,9}-derived line), IAR1902 SCN was evaluated under the experimental ID number of AR09-191018, in the SCN Uniform Soybean Tests.

Statistical Analysis

SAS statistical software (SAS Institute, 2000) was used to run ANOVA and Fisher’s protected LSD on plot data. The genotype × environment interaction variance was considered as the

Table 1. Average soybean cyst nematode (SCN) resistance, iron deficiency chlorosis scores on calcareous soils, and sudden death syndrome disease resistance of soybean cultivar IAR1902 SCN and check cultivars evaluated in the SCN Uniform Soybean Tests, Northern Region in 2010, 2011, and 2012.

Cultivar	Illinois SCN screening†				Iron deficiency chlorosis score‡			SDS§
	HG type 0 (race 3)		HG type 2.5.7 (race 1)		MN	IA, ISU		SIU
	FI	Rating	FI	Rating	Danvers	Dairy	Bruner	DX
	————— 1–5 —————							
	2010							
IAR1902 SCN	1	HR	2	R	2.5	2.9		
Sheyenne	44	LR	86	NR	1.8	2.3		
MN1410	95	NR	80	NR	3.0	3.8		
IA1022	5	HR	80	NR	2.5	3.0		
A11						1.3		
Dwight						3.2		
Average¶	20		58		3.0	3.0		
	2011							
IAR1902 SCN	1	HR	0	HR	2.3	1.8		11
Sheyenne	71	NR	72	NR	2.3	2.0		7
MN1410	68	NR	60	NR	2.3	1.5		19
IA1022	6	HR	61	NR	2.5	2.0		24
A11						1.6		
Dwight						2.0		
M00-456052								1
M97-35718								10
Average¶	13		57		3.0	2.0		8
	2012							
IAR1902 SCN	1	HR	2	HR	2.3	2.3	1.8	
Sheyenne	65	NR	62	NR	1.6	1.8	1.0	
A11						1.2	1.0	
Dwight						2.8	2.7	
Average¶	22		62		3	2	2	

† HG, *Heterodera glycines*; FI = female index; HR = highly resistant (FI < 10), R = resistant (FI 10–24), MR = moderately resistant (FI of 25–39), LR = low resistance (FI 40–59), NR = no effective resistance (FI > 60).

‡ ISU, Iowa State University. Score: 1 = no yellowing, 2 = slight yellowing, 3 = moderate yellowing, 4 = intense yellowing, 5 = severe yellowing with some leaf mortality.

§ SDS = sudden death syndrome; SIU, Southern Illinois University; DX = SDS disease index = (DI × DS)/9, where DI = disease incidence is percentage of plants in plots with visible leaf symptoms and DS = disease severity, rated from 1 = 1–10% of the leaf surface chlorotic or 1–5% necrotic; 2 = 11–20% of the leaf surface chlorotic or 6–10% necrotic; 3 = 21–40% of the leaf surface chlorotic or 11–20% necrotic; 4 = 41–60% of the leaf surface chlorotic or 21–40% necrotic; 5 = greater than 60% chlorotic or greater than 40% necrotic; 6 = up to 1/3 premature defoliation; 7 = 1/3 to 2/3 premature defoliation; 8 = greater than 2/3 premature defoliation; and 9 = plant death before normal defoliation due to senescence. SDS rated only on plants showing symptoms.

¶ Average of all genotypes (entries) in the test.

error variance for traits in the SCN Uniform Tests (P. Dixon, personal communication, 2010). Fisher's protected LSDs were calculated for regional yield trials by summing individual location estimates of error variances from each location's 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. Seed traits were scored by bulking six individual samples per genotype, and therefore no statistical analysis was conducted.

Molecular Analysis

Molecular analyses were performed at the Cianzio laboratory. A polymerase chain reaction (PCR)-based simple sequence repeat (SSR) molecular marker analysis was conducted to detect the presence of SCN and BSR resistance quantitative trait loci (QTL) alleles in the soybean lines.

The Satt309 molecular marker linked to *rhg1*, the major SCN resistance QTL on Chromosome 18 (Chang et al., 2011; Concibido et al., 2004; Cregan et al., 1999; Glover et al., 2004) was used. Satt309 is an important polymorphic marker used to differentiate whether the SCN resistance *rhg1* allele is from Peking or PI 88788 or any other source (Cregan et al., 1999).

Similarly, to detect the presence of BSR resistance QTLs (*Rbs*), SSR molecular markers were used. The BSR resistance parent IAR2001 BSR derives its resistance to BSR from the PI 84946-2 soybean line. The PI 84946-2 was reported to have two major BSR resistance QTLs, *Rbs1* and *Rbs3* (Lewers et al., 1999). The two polymorphic SSR markers linked to the *Rbs1* locus were BARCSOYSSR_16_1130 (BARC_1130) and BARCSOYSSR_16_1131 (BARC_1131) (Klos et al., 2000; Lewers et al., 1999). The two polymorphic SSR markers linked to the *Rbs3* locus were Satt244 and Satt547 (Bachman et al., 2001; Klos et al., 2000; Lewers et al., 1999; Patzoldt et al., 2005; Perez et al., 2010). Sequences of the molecular markers were obtained from the Soybase database (<http://www.soybase.org/>).

Genomic DNA was isolated according to the hexadecyltrimethylammonium bromide (CTAB) extraction method (CIMMYT, 2005) from leaf samples of 10 plants per genotype. The final DNA pellet was resuspended in 100 μ L of 10 mM Tris buffered to pH 8.0 and stored at -20°C until final use. DNA quantity and quality were checked with a Nanodrop spectrophotometer (Thermo Fisher Scientific Inc.). The DNA was diluted with sterile water, and a 20 ng sample was used as a template for PCR. The PCR amplification was performed at 10 μ L volume using a thermal cycler program of 1 min at 94°C ,

Table 2. Average yield and agronomic performance of soybean cultivar IAR1902 SCN and check cultivars evaluated in the soybean cyst nematode (SCN) Uniform Soybean Tests, Northern Region in 2010, 2011, and 2012, on SCN-infested and noninfested soil.

Cultivar	Yield				Maturity date	Lodging	Plant height
	SCN-infested		Noninfested				
	kg ha ⁻¹	Rank	kg ha ⁻¹	Rank		1–5†	cm
2010 24 genotypes (entries) including checks							
No. of locations	10		0		8	9	7
IAR1902 SCN	4315	1			18 Sept.	1.9	98
Sheyenne	2894	24			7 Sept.	1.3	73
MN1410	3698	18			13 Sept.	1.7	83
IA1022	4007	8			18 Sept.	1.9	80
Average‡	3792				17 Sept.	1.8	1.8
Average of checks	3531				13 Sept. 13	1.6	78.25
LSD (0.05)	752				2	0.3	5.7
2011 16 genotypes (entries) including checks							
No. of locations	12		3		11	11	11
IAR1902 SCN	3652	2	3518	5	26 Sept.	1.6	93
Sheyenne	2700	16	3189	13	13 Sept.	1.4	78
MN1410	3062	15	3176	14	18 Sept.	1.6	88
IA1022	3531	5	3330	9	27 Sept.	1.8	85
Average‡	3350		3357		20 Sept.	1.6	83
Average of checks	3095		3239		19 Sept.	1.6	83
LSD (0.05)	743		1972		1.8	0.3	6
2012 10 genotypes (entries) including checks							
No. of locations	11		2		9	10	9
IAR1902 SCN	3538	2	3631	4	18 Sept.	1.5	98
Sheyenne	2171	10	3136	10	6 Sept.	1.2	83
MN1410	2660	8	3463	5	13 Sept.	1.4	85
IA1022	3571	1	3866	1	18 Sept.	1.9	95
Average‡	3035		3491		15 Sept.	1.4	88
Average of checks	2801		3487		12 Sept.	1.5	87
LSD (0.05)	734		2415		2	0.3	6

† 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 50% of the plants down, 4 = all plants leaning considerably or 50 to 80% of the plants down, 5 = all plants down or prostrate.

‡ Average includes the total number of genotypes (entries) in the tests.

40 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 30 s, and extension at 72°C for 30 s. A 10-min extension at 72°C followed the last cycle. The PCR reaction mixtures included 2 mM MgCl₂, 0.25 μM each of forward and reverse primer (Integrated DNA Technologies, Inc.), 2 μM deoxyribonucleotides (dNTPs), and 0.5 U Taq DNA polymerase (Invitrogen, Life Technologies Inc.).

The PCR was done with an ICycler machine (BioRad Inc.), and the amplified PCR products were resolved on a 4% agarose gel along with a 100-bp DNA ladder (NEB Inc.), by running at 120 V for 4 to 7 h. The ethidium bromide-stained PCR products were visualized and pictured following illumination with ultraviolet light.

For the *Rhg4* locus haplotype study, a PCR was performed with two sets of primers flanking the two polymorphic single-nucleotide polymorphisms of the serine hydroxymethyl transferase (*SHMT*) gene at the *Rhg4* locus that governs SCN resistance (Liu et al., 2012). The two single-nucleotide polymorphisms, 389 G/C and 1165 T/A, were PCR amplified by the primers Rhg4-1F (5'-gtcaacgtccagccctactc-3') + Rhg4-1R (5'-tagtctgatgtagccggtggtg-3') and Rhg4-2F (5'-gtgggatctgagacctcttg-3') + Rhg4-2R (5'-gttaccattcgactccacca-3'), respectively. The amplified PCR products were run on 1.2% agarose gel, the correct size bands were excised out, and gel was eluted by columns (Qiagen Inc.) to get the purified DNA. The DNA was submitted for Sanger sequencing by using the forward primers at the Iowa State University DNA facility.

The copy number of the *rhg1* locus was estimated at the Cianzio laboratory using a modified method developed using the quantitative PCR (qPCR) principles described earlier (Cook et al., 2012; Lee et al., 2015). The modified qPCR assay uses *rhg1* locus specific primers and the *UNK2* reference gene specific primers, which were described previously (Cook et al., 2012). qPCR was performed with GoTaq qPCR Master Mix containing higher amplification efficiency BRYT Green Dye (Promega). Genomic DNA was extracted from IAR1902 SCN, Agripro 97284, and IAR2001 BSR and used for qPCR. In addition, the DNA extracted from three known *rhg1* copy number soybean accessions PI 88788 (9 copies of *rhg1*), Peking (3 copies of *rhg1*) and Williams 82 (1 copy of *rhg1*) were used as standards for the copy number estimation. MX3000P qPCR instrument (Stratagene) with a thermal cycler program of 2 min at 95°C hot-start activation, 40 cycles of denaturation at 95°C for 15 s, and annealing/extension at 60°C for 60 s was used. The melting curve was obtained by running one cycle at 95°C for 60 s, 55°C for 30 s, and 95°C for 30 s followed the last cycle. The delta-delta Ct formula was used to calculate the copy number of samples as described previously (Livak and Schmittgen, 2001).

Seed Purification

Seed purification of IAR1902 SCN, as experimental line AR09-191018, was initiated at Ames in 2011 by harvesting 60 F_{4,9} individual plants uniform in agronomic type, maturity, and seed traits. In 2012, F_{9,10} progeny rows of AR09-191018 were grown at Ames to continue seed purification and seed increase processes. Progeny rows with uniform characteristics were separately harvested, and hilum color was checked during winter 2013. Seed of the progeny rows, uniform in agronomic and seed traits, including hilum color, was bulked to produce breeder seed.

On 17 May 2013, F_{9,11} seed harvested from the progeny rows in 2012 was used by the Committee for Agricultural Development at ISU to plant 1.4 ha (3.6 acres) for foundation seed production. Additional disease (BSR) and pest (SCN) screenings were also conducted at the Cianzio laboratory in 2013.

Characteristics

Yield and Agronomic Performance

IAR1902 SCN (tested as experimental line AR09-191018) was evaluated in 2009 in Iowa, in replicated tests at two SCN-infested locations, Mason City and Arlington (Table 3). IAR1902 SCN yield averaged from the two locations was significantly ($P < 0.05$) higher than the high-yielding public checks 'Parker' (Orf and Kennedy, 1994) and 'IA2052' (Fehr, personal communication, 2007). At Mason City, IAR1902 SCN yielded higher than all checks in the test except 'IA1008' (Fehr, personal communication, 2000). At Arlington, IAR1902 SCN

Table 3. Average yield and agronomic performance of soybean cultivar IAR1902 SCN and high-yielding check cultivars evaluated in 2009 in two soybean cyst nematode (SCN)-infested locations in Iowa, Mason City and Arlington. Two replications of two-row plots were planted at each location.

Cultivar	Yield kg ha ⁻¹	Maturity	Lodging score†
2009			
Mason City, IA			
IAR1902 SCN	4087	25 Sept.	2.2
Parker	3975	27 Sept.	2.8
IA1008	5561	29 Sept.	2.0
IA2052	3678	30 Sept.	2.0
IA2068	3819	2 Oct.	2.0
Loda	3578	29 Sept.	2.3
Average‡	3551	28 Sept.	2.6
LSD (0.05)	622	3.2	0.81
CV	8.81	5.89	15.89
Arlington, IA			
IAR1902 SCN	4054		2.3
Parker	2835		3.8
IA1021	2988		2.3
BSR101	3116		2.5
IA2052	3672		2.5
LD02-4485	3564		2.5
Average‡	3451		2.7
LSD (0.05)	377		0.56
CV	5.51		10.71
Average across locations			
IAR1902 SCN	4067		2.3
Parker§	3008		3.3
IA2052§	3745		2.3
Average‡	3497		2.6
LSD	452		0.58
CV	9.27		15.97

† 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 50% of the plants down, 4 = all plants leaning considerably or 50 to 80% of the plants down, 5 = all plants down or prostrate.

‡ Average includes a total of 43 entries or genotypes.

§ Planted at each of two locations.

surpassed in yield all of the public check cultivars that were included in the test.

From 2010 to 2012, IAR1902 SCN was evaluated in the SCN Uniform Soybean Tests (Table 2) under the experimental designation AR09-191018. In 2010, all tests were conducted on SCN-infested soil, and IAR1902 SCN ranked first of all the 24 entries in the test. Ranking of IAR1902 SCN ranged at individual locations from number two to nine (data not shown), in all cases being the highest-yielding experimental line of the check cultivars included in the tests. In 2011 and 2012, tests were planted on SCN-infested and noninfested locations, with the majority of the tests planted on SCN-infested locations (Table 2). In 2011, IAR1902 SCN ranked 2 of 16 genotypes, and in 2012 it was ranked 2 of 10 genotypes. The ranking of IAR1902 SCN in 2011 at individual locations ranged from 1 to 12, always higher yielding than the public check cultivars included in the tests (data not shown). In 2012, IAR1902 SCN yield was ranked first in the majority of the individual locations, with six being its lowest rank (data not shown). On the noninfested soil, IAR1902 SCN ranked five in 2011 and four in 2012 (Table 2). During 2011 and 2012, the same high-yielding checks were planted at both SCN-infested and noninfested conditions.

IAR1902 SCN is classified as MG I (maturity subgroup 1.9) (Tables 2 and 3). In Mason City, Iowa, its maturity ranged from two to 7 d earlier than the checks and 2 d earlier than Parker, the earliest maturity check in the test (Table 3). On that same test, it matured 7 d earlier than IA2068, the latest maturity check of the group. In the SCN Uniform Regional Test conducted in 2010, IAR1902 SCN matured 5 d later than MN1410 and was of the same maturity as that of IA1022 (Table 2). In 2011, IAR1902 SCN matured 1 d earlier than IA1022 and 8 d later than MN1410. Similar variation among the three genotypes was observed in the 2012 tests. Lodging scores of IAR1902 SCN were similar to the public cultivars in the tests, with some variation due to year, ranging from 1.5 to 2.3 (Tables 1 and 2). Plant height of IAR1902 was similar from 2010 to 2012 and always taller than the high-yielding checks (Table 2).

Soybean Cyst Nematode Resistance

IAR1902 SCN was classified as highly resistant to HG type 0 at the SCN screenings conducted at Illinois and resistant and/or highly resistant to HG type 2.5.7 (Table 1). Compared with public high-yielding check cultivars included in the tests, the IAR1902 SCN cultivar was either superior or similar in resistance to HG type 0 and always superior in resistance to the HG type 2.5.7 among the check cultivars.

The screening of the 60 individual plants of IAR1902 SCN conducted during the seed purification process identified it as being resistant to the HG type 0-infested soil collected from the Muscatine location in Iowa (Table 4). In the tests, parents of the line and the cultivars ‘Jack’ (Nickell et al., 1990), BSR 101, and ‘Williams 82’ (Bernard and Cremeens, 1988) were also included. The Agripro parent was rated as highly resistant to HG type 0.

Brown Stem Rot Resistance

Seed of the 60 individual plants that were reevaluated for SCN resistance during purification was also evaluated for BSR

resistance (Table 4). IAR1902 SCN had a slightly better BSR foliar severity score than the IAR2001 BSR resistant parent and had a score very close to that of the BSR resistant cultivar BSR 101. Williams 82 had the lowest score of the test.

Iron Deficiency Chlorosis and Sudden Death Syndrome Evaluations

IAR1902 SCN was also screened for IDC and SDS resistance at the SCN Uniform Soybean Tests evaluations (Table 1). Iron deficiency chlorosis was screened on calcareous soil at one location in Minnesota and two locations in Iowa from 2010 to 2012. Average IDC scores were adequate at the Minnesota location, with a score ranging from 2.3 to 2.5, depending on the year. In Iowa, one location was used in 2010 and 2011, and two locations were used in 2012. IAR1902 SCN had IDC scores that were acceptable in 2011 and at the two locations in Iowa during 2012, comparable to the scores assigned in Minnesota. In 2010, the IDC score at the Iowa location was borderline to being not acceptable.

The SDS disease severity was scored at Southern Illinois University during 2011 (Table 1). IAR1902 SCN had a disease index of 11, indicating that the foliar symptoms due to this disease were acceptable. Note that this is based on only 1 yr of data, for a disease that is highly dependent on the environment (Leandro et al., 2013).

Table 4. Average soybean cyst nematode (SCN) resistance in greenhouse conditions and brown stem rot (BSR) resistance in growth chamber conditions for soybean cultivar IAR1902 SCN and check cultivars at the Cianzio laboratory, Iowa State University. Screening for both diseases was conducted using individual plants of IAR1902 SCN that were uniform for agronomic traits.

Cultivar	SCN greenhouse screening†		BSR growth chamber screening‡
	No. of cysts	Rating	1–7
IAR1902 SCN	18	R	5.9
Parents			
IAR2001 BSR	42	LR	5.0
Agripro 97284-N00-47977	4	HR	5.7
Checks			
Jack (SCN resistant)	0	HR	
BSR101 (BSR resistant)			6.0
Williams 82 (SCN susceptible)	49	LR	3.7
Average§	23	MR	5.3
LSD (0.05)	19		1.3
CV	26		13

† Soil infested with SCN HG type 0 (race 3). Cysts were counted on the outside of each root ball, after cysts were identified on cultivar Williams 82. The test was conducted on each of 120 individual plants of IAR1902 SCN and check cultivars planted in two replications, one plant per container. HR = highly resistant (<10 cysts), R = resistant (10–20 cysts), MR = moderately resistant (25–39 cysts), LR = low resistance (40–59 cysts), and NR = no effective resistance =>60 cysts). Jack traces resistance to PI 88788.

‡ Foliar severity: 1 = dead plant, 2 = plant with green stem and no leaves, 3 = chlorotic and necrotic leaves are prominent, 4 = plants stunted, mosaic chlorosis and necrosis on leaves, 5 = leaf area appears normal except for some yellowing, 6 = normal leaf area except for some yellowing, 7 = leaf area is normal and plants are small but healthy, 7 = plants are completely normal.

Botanical Description and Seed Traits

IAR1902 SCN of MG I (maturity subgroup 1.9), has purple flowers, gray pubescence, tan pods, seed with buff hila, yellow seed coat, and dull seed coat luster. Seed weight, quality, and protein and oil content were evaluated from 2010 to 2012 at the SCN Uniform Soybean Tests (Table 5). Seed weight of IAR1902 SCN was similar over years and also similar to that of the check cultivars included in the tests. Seed quality of IAR1902 SCN was similar among years, having heavier seeds in 2012. Compared with the checks, seed weight was either similar or slightly lighter than the check seed at each of the 3 yr. Seed quality of IAR1902 SCN in 2010 and 2011 was consistently superior to the seed quality of Sheyenne and was either similar or slightly better than all other checks in the tests. Protein and oil content were similar among the 3 yr, with only minor variations. The protein and oil content of IAR1902 SCN was comparable to that of seed of check cultivars included in the test.

Table 5. Average seed size (seed weight), seed quality, and seed composition (protein and oil percentages) of soybean cultivar IAR1902 SCN and check cultivars evaluated in the Soybean Cyst Nematode (SCN) Uniform Soybean Tests, Northern Region in 2010, 2011, and 2012.

Cultivar	Seed			
	Size†	Quality‡	Protein§	Oil§
	g 100 seed ⁻¹	1–5	%	%
2010, 24 entries including checks				
IAR1902 SCN	14.3	1.3	34.8	18.7
Sheyenne	15.1	2.3	34.5	18.5
MN1410	15.5	1.8	35.8	18.2
IA1022	14.3	1.2	34.3	19.2
Average¶	14.3	1.5	35.0	18.1
Average of checks	15.0	1.8	34.9	18.6
LSD (0.05)	0.6	0.5	0.6	0.5
2011, 16 entries including checks				
IAR1902 SCN	14.5	1.5	34.2	19.0
Sheyenne	14.8	2.3	34.5	18.8
MN1410	15.1	1.8	35.0	18.9
IA1022	14.5	1.5	32.3	19.5
Average¶	14.9	1.8	34.3	18.6
Average of checks	14.8	1.9	33.9	19.1
LSD (0.05)	0.9	0.5	0.7	0.4
2012, 10 entries including checks				
IAR1902 SCN	15.4	1.3	34.3	19.7
Sheyenne	14.6	1.6	34.4	19.1
MN1410	15.9	1.3	36.0	18.6
IA1022	15.5	1.2	33.0	19.9
Average¶	15.6	1.4	35.0	18.8
Average of checks	15.3	1.4	34.5	19.2
LSD (0.05)	0.7	0.3	0.6	0.4

† Seed size recorded as the weight in g 100 seed⁻¹ based on a 100- or 200-seed sample.

‡ Seed quality rated according the following scores, considering amount and degree of wrinkling, defective seed coat (growth cracks), greenishness, and moldy or rotten seeds. Threshing or handling damage is not included, nor is mottling or other pigments. 1 = very good, 2 = good, 3 = fair, 4 = poor, and 5 = very poor.

§ Composition was measured at the University of Minnesota by infrared reflectance, reported as dry-weight percentage converted to a 13% moisture basis.

¶ Average of all genotypes (entries) in the tests.

Molecular Analysis Results for Soybean Cyst Nematode and Brown Stem Rot Resistance

The *rhg1* locus on Chromosome 18 has been mapped from various sources (Concibido et al., 2004). Previous studies showed that there are two different types of *rhg1*: the Peking-type *rhg1-a* and the PI 88788-type *rhg1-b* (Concibido et al., 2004). PI 88788-type *rhg1-b* can confer high levels of SCN resistance by itself (Cook et al., 2012; Kim et al., 2010; Yu et al., 2016) and typically has a high copy number (nine copies of the *rhg1* repeat). In contrast, the Peking-type *rhg1-a* requires *Rhg4* to confer full resistance (Liu et al., 2012; Meksem et al., 2001), and it has three copies of the *rhg1* repeat (Cook et al., 2012; Lee et al., 2015).

For the region where *rhg1* maps on Chromosome 18, the polymorphic Satt309 marker was used to determine the nature of SCN resistance for IAR1902 SCN. The DNA band size of the PCR products of IAR1902 SCN and the Agripro line were similar to Peking (Fig. 1). This indicates that the IAR1902

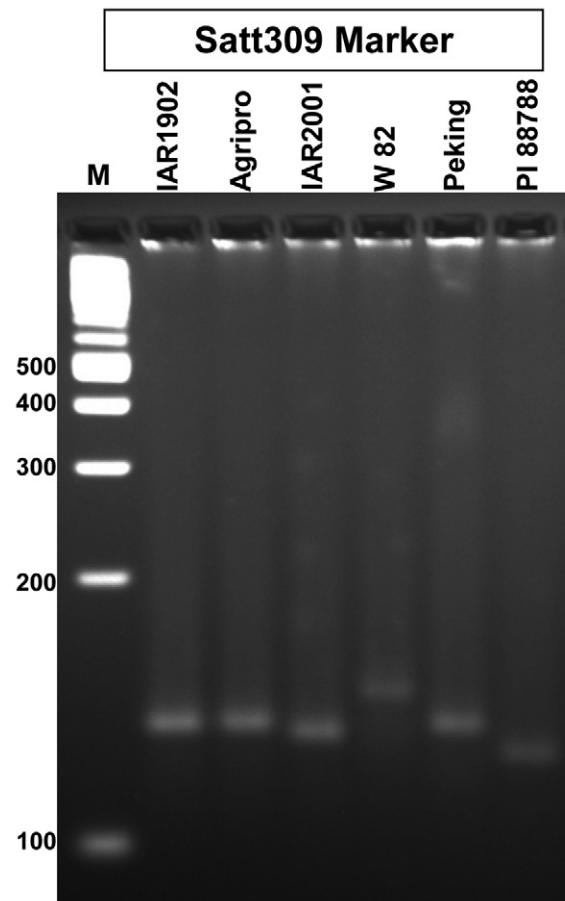


Fig. 1. Quantitative trait loci associated with soybean cyst nematode (SCN) resistance. Agarose gel photograph of polymerase chain reaction (PCR) products of IAR1902 SCN (IAR1902), Agripro 97284-N00-47977 (Agripro), IAR2001 BSR (IAR2001), Williams 82 (W 82), Peking, and PI 88788 genotypes with Satt309 molecular marker. Ten replications were pooled in 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. Satt309 marker is associated with *rhg1* locus and was polymorphic among the parents. The result clearly show that the resistance allele *rhg1* in IAR1902 SCN was inherited from Peking via the Agripro parent.

SCN line inherited the most important SCN resistance *rhg1-a* allele from the SCN-resistant parent Agripro, which in turn received it from the Peking resistance source. It is also evident that no segregation is occurring for the SCN resistance, indicating that the trait is fixed. Copy number estimate showed that IAR1902 SCN and the Agripro line inherited three copies of the *rhg1-a* locus from the Peking source (Fig. 2). The results confirmed that the Peking line has three copies of the *rhg1* gene and PI 88788 has nine copies of *rhg1*. Further, the haplotype characterization to determine the *Rhg4* (*SHMT*) nucleotide sequence polymorphism showed that IAR1902 SCN and Agripro inherited the Peking-type SCN resistance *Rhg4* haplotype (Table 6).

The PCR band size of IAR2001 BSR is not only different from the SCN susceptible Williams 82 but also different from other soybean lines (Fig. 1). The haplotype results confirmed that IAR2001 BSR, PI 88788, and Williams 82 inherited similar *Rhg4* haplotype (Table 6). However, the qPCR results showed that IAR2001 BSR has one *rhg1* copy as that of Williams 82, whereas PI 88788 has nine copies. Recent greenhouse assay confirmed that IAR2001 BSR was susceptible to SCN HG type 2.5.7 (data not shown). The results indicate that qPCR *rhg1* copy number estimation must be performed in addition to

PCR and haplotype characterization to determine the nature of SCN resistance of a soybean genotype at molecular level.

To detect the presence of BSR resistance QTLs (*Rbs*), SSR molecular markers were used. The BSR resistance parent IAR2001 BSR, derives its resistance to BSR from the PI 84946-2 line. PI 84946-2 was reported to have two major BSR resistance QTLs, *Rbs1* and *Rbs3* (Lewers et al., 1999). The PCR analysis with the *Rbs1* and *Rbs3* linked molecular markers clearly showed that DNA banding pattern of IAR1902 SCN, IAR2001 BSR, and PI 84946-2 are similar to each other (Fig. 3), whereas, the DNA banding pattern of Agripro and BSR-susceptible Williams 82 match each other. The results demonstrate that the IAR1902 SCN inherited both the major BSR resistance loci (*Rbs1* and *Rbs3*) from the parent IAR2001 BSR, which traces to PI 84946-2.

Conclusions

The soybean cultivar IAR1902 SCN was released for its high yield and resistance to SCN derived from the Peking SCN-resistant source. The resistance to SCN derived from Peking makes it one of the few public conventional available cultivars possessing that source. IAR1902 SCN is of MG I (mid to late within the MG), similar in maturity to IA1022 of MG I and about 8 d later than Sheyenne of MG 0 (relative maturity 0.8). Yield of IAR1902

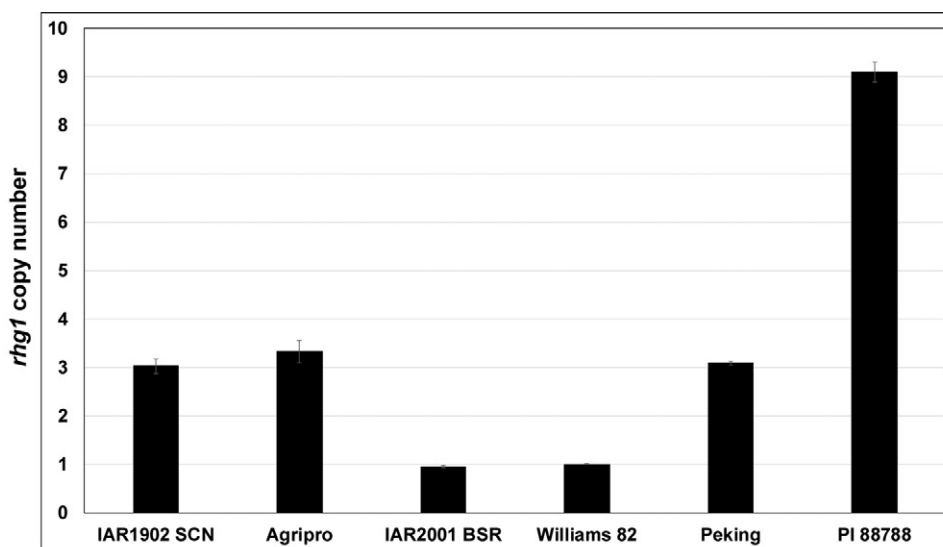


Fig. 2. Quantitative polymerase chain reaction (qPCR) assay of copy number analysis of *rhg1* locus of different genotypes. The copy number estimation of *rhg1* locus different genotypes, IAR1902 SCN (three copies), Agripro 97284-N00-47977 (three copies), IAR2001 BSR (one copy), including the known *rhg1* copy number checks, Williams 82 (one copy), Peking (three copies), and PI 88788 (nine copies). From the qPCR results, the copy number estimate values were obtained for each genotypes using the delta-delta Ct formula (Livak and Schmittgen, 2001), and the values were represented in the form of a bar chart. The bar represents 10 biological replicates and 3 technical replicates of *rhg1* copy number with their standard error. The result clearly show that the resistance allele *rhg1* in IAR1902 SCN was inherited from Peking via the Agripro parent.

Table 6. Single-nucleotide polymorphism haplotype of *Rhg4* (serine hydroxyl methyl transferase enzyme or *SHMT*) locus.

Soybean line	<i>SHMT</i> 389 G/C†	<i>SHMT</i> 1165 T/A†	<i>Rhg4</i> haplotype	Phenotype‡
IAR1902 SCN	G	T	Peking-type	SCN resistance
Agripro 97284	G	T	Peking-type	SCN resistance
IAR2001 BSR	C	A	Williams 82-type	SCN susceptible
Williams 82	C	A	–	SCN susceptible
Peking	G	T	–	SCN resistance
PI 88788	C	A	–	SCN resistance

† Polymerase chain reaction (PCR) was carried out to amplify the polymorphic region of serine hydroxylmethyl transferase (*SHMT*) and Sanger sequencing of the PCR products was carried out to find out the polymorphism of the soybean lines.

‡ SCN, soybean cyst nematode.

SCN is superior to current public cultivars included in the tests that were also SCN resistance. IAR1902 SCN is adapted to soybean fields in the northern soybean production region, 40° to 42° N latitude, and highly resistant to SCN HG type 0 and either highly resistant or resistant to HG type 2.5.7. It is also resistant to BSR, having BSR scores similar to IAR2001 BSR. IAR1902 SCN has an IDC score comparable to, and in the majority of the cases better than, other public cultivars. It also has acceptable SDS disease severity scores on the basis of a 1-yr test. Molecular marker

analysis revealed that IAR1902 SCN inherited the major SCN resistance *rhg1-a* allele from Peking via the SCN-resistant Agripro parent. Additional molecular marker analysis confirmed that the two major BSR resistance QTL, *Rbs1* and *Rbs3*, were inherited by IAR1902 SCN from PI 84946-2 via the IAR2001 BSR parent. IAR1902 SCN will serve in production conditions in which SCN is prevalent and BSR may also be a threat, and where the SDS disease and the abiotic stress factor IDC might also be present.

Availability

Seed of IAR1902 SCN for research and breeding purposes may be obtained directly from Iowa State University by contacting Silvia R. Cianzio, and ISURF, requesting seed for IAR1902 SCN (ISURF Docket # 04340). A Material Transfer Agreement (MTA) will be signed between parties, after which seed will be made available. Seed of cultivar IAR1902 SCN has also been deposited in the USDA-ARS National Plant Germplasm System, where it will be available 20 years from the date of publication. The cultivar may be available for branding, as per negotiations accorded on the MTA, among ISURF, Silvia R. Cianzio, and the interested party.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

The line was developed with funds provided by the Iowa Soybean Association and the United Soybean Board., ISU project number 4403. Funds were all assigned to the ISU Soybean Breeding Project for yield and introgression of defensive traits. Additional funds were provided by the Agriculture and Home Economics Experiment Station, Iowa State University, Ames, IA 50011-1010. J.P.S. Carvalho, the visiting scholar from Brazil to Cianzio laboratory at ISU was financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior- Brasil, Finance code 001. Appreciation is extended to Mr. Troy Cary, Mr. Gary Knowing, and Dr. Anne Gillian, 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 Dr. J. Bond, coordinator of the SDS Uniform Regional Tests, for his work, attention to detail, and collaboration in preparing and summarizing data corresponding to the tests. Data summarized by Dr. Bond and Mr. Cary have been 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.

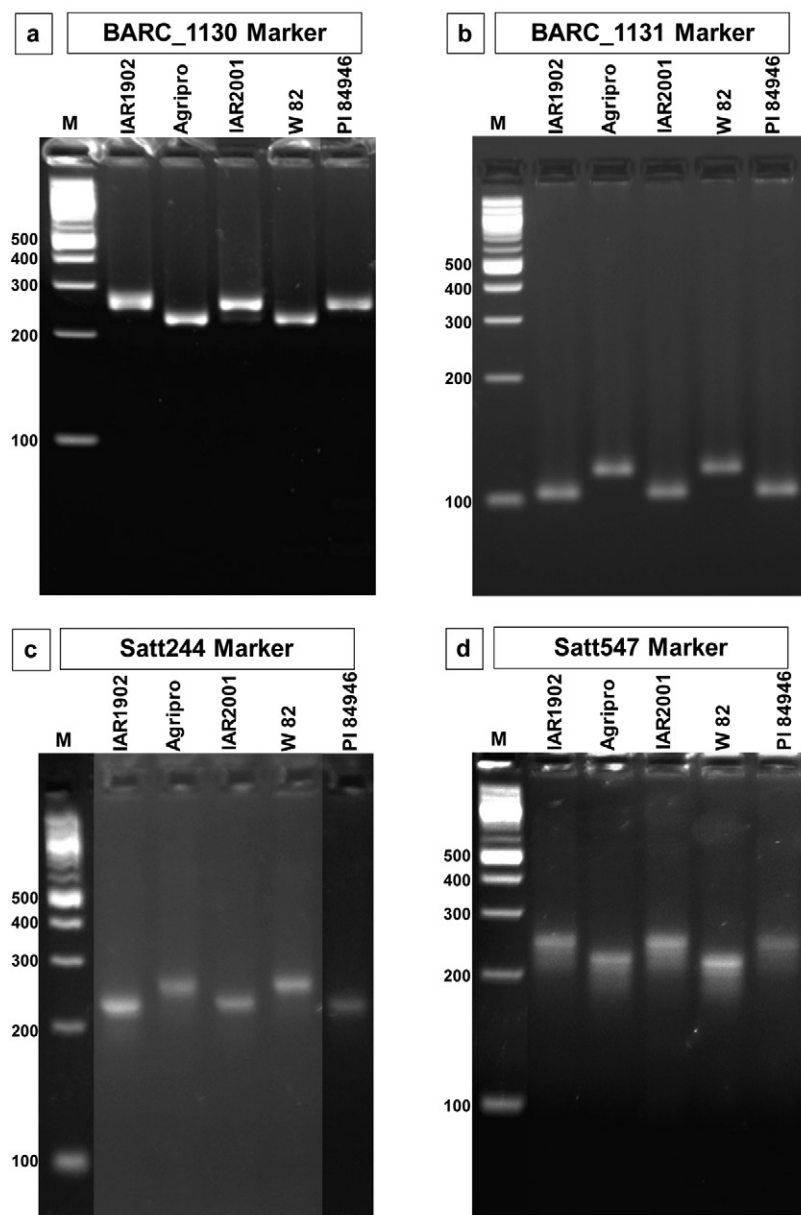


Fig. 3. Quantitative trait loci (QTL) associated with brown stem rot (BSR) resistance. Agarose gel photograph of polymerase chain reaction (PCR) products of IAR1902 SCN (IAR1902), Agripro 97284-N00-47977 (Agripro), IAR2001 BSR (IAR2001), Williams 82 (W 82), and PI 84946-2 genotypes with different molecular markers. Ten replications were pooled in 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. Markers (a) BARCSOYSSR_16_1130 (BARC_1130) and (b) BARCSOYSSR_16_1131 (BARC_1131) are associated with the BSR resistance *Rbs1* QTL and were polymorphic among the parents. The *Rbs1* locus in IAR1902 SCN was inherited from the IAR2001 BSR parent. Markers (c) Satt244 and (d) Satt547 are associated with the BSR resistance *Rbs3* QTL and were polymorphic among the parents. The *Rbs3* locus in IAR1902 SCN was inherited from the IAR2001 BSR parent. The result clearly show that both the *Rbs1* and *Rbs3* resistance alleles in IAR1902 SCN were inherited from PI 84946-2 via the IAR2001 BSR parent.

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