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ORIGINAL PAPER

F. J. Kopisch-Obuch · B. W. Diers

Segregation at the SCN resistance locus *rhg1* in soybean is distorted by an association between the resistance allele and reduced field emergence

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Abstract Segregation distortion has been reported repeatedly in soybean (*Glycine max* [L.] Merr.) inbred line populations segregating for the soybean cyst nematode (SCN) (*Heterodera glycines* Ichinohe) resistance gene *rhg1*. In each reported case, the frequency of the SCN resistance allele at the *rhg1* locus was lower than expected. Segregation distortion was studied in 51 F_4 populations by counting the number of plants predicted to be homozygous resistant, susceptible, and heterozygous for *rhg1* based on the genetic markers Satt309, CTA, or TMA5. Significant ($P < 0.05$) segregation distortion was observed in 44 out of the 51 F_4 populations. When the heterozygotes were ignored, there were significantly fewer homozygous-resistant plants than expected in 33 populations. To study whether differential field emergence was a cause of the segregation distortion, three near isogenic line (NIL) populations segregating at the *rhg1* locus for SCN resistance from plant introduction 88788 were tested. Population sizes ranged from 32 to 44 NILs and emergence was determined in field experiments in three environments. In each population, SCN-resistant NILs had significantly ($P < 0.05$) less field emergence than susceptible NILs. In the population with the greatest effect, field emergence of resistant NILs was 6% less than susceptible NILs, with the entire population having an average emergence rate of 46%. Equations were derived to describe the effect of selection on segregation ratios over generations of population development and the observed emergence rates were transformed into fitness factors. Depending on assumptions of gene action, it was predicted from these fitness factors that segregation distortions were in the range of those reported previously for the *rhg1* locus and were similar to what was observed on average across the 51 F_4 populations. While other factors might also be involved, the results suggest that reduced field emergence associated with the SCN resistance allele contributes to previously reported segregation distortion at the *rhg1* locus.

Abbreviations AFLP: Amplified fragment length polymorphism · ANOVA: Analysis of variance · cM: centiMorgan · LG: Linkage group · NIL: Near isogenic line · PCR: Polymerase chain reaction · PI: Plant introduction · QTL: Quantitative trait loci · SCN: Soybean cyst nematode

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F. J. Kopisch-Obuch
Institute of Agronomy and Plant Breeding,
Georg-August-University, Von-Siebold-Str. 8,
37075 Göttingen, Germany

B. W. Diers (✉)
Department of Crop Sciences, University of Illinois,
1101 W. Peabody Dr., Urbana, IL, 61801 USA
E-mail: bdiers@uiuc.edu

Introduction

The incorporation of favorable agronomic traits from non-adapted exotic germplasm into elite breeding lines can be accompanied by the introgression of unfavorable genes due to linkage drag or pleiotropic associations (Jensen 1988). In a recent field study, Kopisch-Obuch et al. (2005) studied whether soybean (*Glycine max* [L.] Merr.) seed yield depression was associated with the major soybean cyst nematode (SCN)-resistance gene *rhg1* from plant introduction (PI) 88788. Under low SCN pressure, this yield depression was significant in one near isogenic line (NIL) soybean population. Association of SCN resistance with plant maturity, plant lodging, and plant height was also detected.

Segregation distortion for genetic markers closely linked to *rhg1* on linkage group (LG) G was observed in populations segregating for susceptibility and resistance alleles from several sources at this locus. This includes segregation distortion resulting in fewer than expected plants or lines that were homozygous for the *rhg1* resistance allele from PI 437654 (Webb et al. 1995), PI

209332 (Mudge et al. 1997), 'Hartwig', which has resistance from PI 437654 and 'Peking' (Prabhu et al. 1999), and PI 88788 (Glover et al. 2004). Distortion was also observed for genetic markers linked to a SCN resistance quantitative trait locus (QTL) on LG M (Webb et al. 1995) and LG A2 (Prabhu et al. 1999). Furthermore, significant interactions between the segregation at the *rhg1* locus and segregation of SCN resistance QTL on LG M (Webb et al. 1995) and LG J (Glover et al. 2004) were observed. In these cases, the segregation distortion at *rhg1* was only apparent in lines that were homozygous for the SCN susceptibility allele on either LGs M or J.

Segregation distortion could be caused by preferential chromosome segregation (Rhoades 1942), preferential gametic selection during fertilization (Schwemmle 1938), or pollen tube competition (Grant 1975). It may also be caused by genes affecting fitness and viability of seeds or plants during germination, seedling emergence, or plant growth. Such viability or fitness genes could be pleiotropic, or linked with the gene controlling the trait with the observed segregation distortion (Grant 1967). Nobs and Hiersy (1957) reported segregation distortion for flower color in a population derived from an interspecific *Mumulus* cross (*M. cardinalis* × *M. lewisii*). Segregation distortion was observed only under increased environmental stress and it was explained by linkage between flower color and a fitness trait affecting seedling mortality. Bradshaw and Stettler (1994) explained segregation distortion of a DNA marker in an interspecific *Populus* cross (*P. trichocarpa* × *P. deltoides*) by linkage between the marker and a locus for a viability trait expressed during embryo development or at the seedling stage.

The objectives of this study were (1) to test for segregation distortion at the *rhg1* locus across different genetic backgrounds and (2) to test whether this segregation distortion can be at least partially explained by an association between *rhg1* and seedling emergence.

Materials and methods

Plant material

Segregation distortion was tested in 51 populations of F_4 plants segregating for SCN resistance at the *rhg1* locus. The number of plants in these populations varied, although there were at least 50 plants in each population. These populations were developed from crossing SCN-resistant and susceptible parents during the summers of 1998 or 1999 in Urbana, IL. All SCN-resistant parents except 'Ina', M90-18111, and LN95-15200-97 had resistance derived solely from PI 88788. Both Ina and LN95-15200-97 have resistance from PI 88788, Peking, PI 437654, and the line M90-184111 has resistance from PI 88788 and PI 209332. In 1999 or 2000, F_1 plants were grown in the field in Urbana, and F_2 populations were planted in November of the same year in a winter nursery in Puerto Rico. A single pod was harvested from each plant and the pods from each population were threshed in bulk and used to plant the F_3 generation the following January. Pod harvesting and threshing was repeated and the F_4 seeds were planted in the field at Urbana during the springs of 2000 or 2001.

Three NIL populations segregating for the major SCN resistance QTL *rhg1* were used to test for an association between SCN resistance and seedling emergence. The development of these populations has been described in detail in a previous article on the association between SCN resistance and yield (Kopisch-Obuch et al. 2005). Populations BR-1 and BR-2 were each developed from a different F_4 plant that was predicted to be heterozygous for SCN resistance at the *rhg1* locus based on genetic markers flanking this locus. The F_4 plants were derived from a cross between 'Bell', which has SCN resistance from PI 88788, and the SCN-susceptible cultivar Colfax. Lines were derived from these F_4 plants and advanced to the F_7 generation in bulk, and the F_7 plants were individually threshed to form F_7-derived lines. BR-1 consisted of 8 homozygous-resistant and 26 homozygous-susceptible NILs, and BR-2 consisted of 22 homozygous-resistant and 22 homozygous-susceptible NILs based on marker data. Both BR populations are fixed for the Bell-derived resistance allele at the SCN resistance QTL cqSCN-003 on LG J. Similar to BR-1 and BR-2, population SR-2 was formed from a F_6 plant predicted to be heterozygous for *rhg1* based on alleles at linked markers. The F_6 plant was from a population developed from a cross between S42-M1, which has SCN resistance from PI 88788, and the SCN-susceptible cultivar S22-C3. A $F_{6:7}$ line was developed from the F_6 plant and NILs were developed by individually threshing the F_7 plants. This population consists of 15 homozygous-resistant and 17 homozygous-susceptible NILs. SR-2 is fixed for the S22-C3-derived susceptible allele at cqSCN-003.

Field experiments

The 51 F4 populations were tested in 2000 or 2001 in Urbana, IL, where Flanagan silt loam (Fine, smectitic, mesic Aquic Argiudolls) was the predominant soil type. Seeds were planted in four-row plots with a 0.76 m row spacing, a 3.2 m length, a seeding rate of 19 seeds m^{-1} row, and at a depth of 3.2 cm. When sufficient seed was available, populations were planted across two plots. Populations were planted on 1 June 2000 and 16 May 2001. Starting at the V5 stage (Fehr et al. 1971), individual plants were genotyped with DNA markers closely linked to *rhg1*.

The NIL populations BR-2 and SR-2 were planted in 2002 as separate randomized complete block design (RCBD) tests with two replications in two locations. In both locations, two-row plots with a 0.76 m row spacing and a seeding rate of 30 seeds m^{-1} row were planted. The St. Joseph, IL location was planted on 24 May in 3.66 m long plots, with a planting depth of 2.5 cm. The

Urbana, IL location was planted on 21 May in 3.2 m long plots, with a planting depth of 3.2 cm. F_{11} seeds harvested in Urbana, 2001, were planted at both locations. In St. Joseph, the predominant soil type was Flanagan silt loam (Fine, smectitic, mesic Aquic Argiudolls) and in Urbana, a Thorp silt loam (Fine-silty, mixed, superactive, mesic Typic Argiaquic Argialbolls). The number of seedlings in each plot was counted at the V2 to V4 stage (Fehr et al. 1971) and reported as percent field emergence.

In 2004, the NILs in BR-1, BR-2 and SR-2 were randomized together in one experiment grown in Urbana. Three replicate blocks of the experiment were planted on 4 May at a seeding rate of 17 seeds m^{-1} row, with planting depth treatments of 3.0 and 6.0 cm. Within each block, plots were arranged in a split plot design with the two planting depths assigned to the macro plots and lines nested within each depth in an alpha lattice incomplete block design (Patterson and Williams 1976). Lines were planted in one-row plots with a 0.76 m row spacing, a 3.2 m length, and a seeding rate of 31.25 seeds m^{-1}. F_{10} seeds harvested in a winter nursery in Kekaha, HI, during the spring of 2001 were planted in this test. The seed had been kept in cold storage from 2001 to 2004. Due to limited seed supply, two homozygous-resistant BR-2 NILs were not planted in 2004. Dana silt loam (Fine-silty, mixed, superactive, mesic Oxyaquic Argiudolls) was the predominant soil type of the first replication, Flanagan silt loam (Fine, smectitic, mesic Aquic Argiudolls) of the second replication, and Drummer silty clay loam (Fine-silty, mixed, superactive, mesic Typic Endoaquoll) of the third replication. Field emergence in the 2004 experiment was determined by counting seedlings at the VE to V2 stages (Fehr et al. 1971), as described earlier.

DNA marker analysis

The segregation ratio of *rhg1* in each F_4 population was determined by genotyping individual F_4 plants in Urbana in 2000 and 2001 with DNA markers linked to *rhg1*. The marker analysis was done by first isolating DNA from each F_4 plant using a quick extraction protocol developed by Bell-Johnson et al. (1998). The DNA was analyzed with the polymerase chain reaction (PCR)-based markers Satt309, CTA, or TMA5, by methods described in Cregan and Quigley (1997). The marker that gave the clearest polymorphism was used in each population. The simple sequence repeat marker (SSR) Satt309 was mapped 0.4 centiMorgan (cM) distal to *rhg1* (Cregan et al. 1999), and the sequence characterized amplified region (SCAR) marker CTA was developed from the amplified fragment length polymorphism (AFLP) marker $E_{CTA}M_{AGG}113$, which is 2 cM proximal to *rhg1* (Meksem et al. 2001a). TMA5 is a sequence-tagged-site marker developed from the AFLP marker $E_{ATG}M_{CGA}87$ (Meksem et al. 2001b), which is located 0.5-cM distal to *rhg1* (Meksem et al. 2001a). PCR products were analyzed in 3% metaphor (FMC BioProducts, Rockland, ME) agarose gels or 6% (w/v) nondenaturing polyacrylamide gels (Wang et al. 2003).

Statistical analysis

Segregation distortion in the F_4 populations for the observed segregation of RR (homozygous for the allele from SCN-resistant parent), RS (heterozygous), and SS (homozygous for the allele from the susceptible parent) for the markers linked to *rhg1* was tested with a chi-square test. Deviation from the expected 7 RR:2 RS:7 SS ratio and for deviation from the expected 1 RR:1 SS ratio in each F_4 population were tested. Similarity of segregation ratios observed in different F_4 populations were tested with a chi-square association test using the FREQ PROCEDURE of SAS (SAS Institute 2000).

The results from the field emergence tests were computed separately for each population in the 2002 experiments and across populations in the 2004 experiment using the MIXED PROCEDURE of SAS (SAS Institute 2000). Locations and NILs were treated as random factors and population and planting depth as fixed. The NIL by location interaction in the analysis of variance (ANOVA) of the 2002 experiments, and the NIL by planting depth interaction in the ANOVA of the 2004 experiments were pooled into the error term if not significant at $\alpha = 0.25$.

Furthermore, ANOVAs were performed to test the genetic effects of the regions containing the SCN-resistance gene by nesting NILs within SCN-resistance allele (resistant or susceptible) with SCN-resistance allele treated as a fixed factor. The SCN-resistance allele by location and NIL × location interaction in the ANOVA of the 2002 experiments, and the NIL × planting depth interaction in the ANOVA of the 2004 experiment were pooled into the errors if not significant at $\alpha = 0.25$. Preplanned contrasts between resistant and susceptible NILs were calculated using the ESTIMATE statement of the MIXED PROCEDURE. Further contrasts were calculated for the 2004 experiments to explain interactions and main effects that were significant at $\alpha = 0.05$.

An ANOVA was also conducted across the 2002 and 2004 experiments if the NIL by location interaction in 2002 and the NIL × planting depth interaction in 2004 were not significant at $\alpha = 0.25$. For the 2004 experiment, the adjusted NIL means within each level of planting depth were used, instead of raw data, treating the experiment as a RCBD with the two planting depth levels as two replications. This ANOVA across years was computed separately for each BR-2 and SR-2.

Effect of field emergence on segregation distortion

The effect of the observed differences between SCN -resistant and susceptible NILs for field emergence on segregation distortion at the *rhg1* locus was esti-

mated. Equations were derived from Hedrick (2005) to describe the effect of selection on segregation ratios over generations of population development through single-seed descent. A fitness factor, w, was determined for homozygous SCN-resistant NILs and for homozygous-susceptible NILs. On the basis of percent field emergence, w ranged from 0 (no individual of the genotypic class contributes to the next generation) to 1 (all individuals of the genotypic class contribute to the next generation). Since field emergence data on lines composed of heterozygous plants were not collected it could not be determined whether greater field emergence was dominant, incompletely-dominant, or recessive. Therefore, the effect of each type of gene action was tested. Genotypic frequencies in advanced inbreeding generations were calculated up to the F_6 generation according to the following equations:

$$P_{F_n} = \frac{w_p(P_{F_{n-1}} + 1/4H_{F_{n-1}})}{\bar{w}_{F_n}},$$

$$H_{F_n} = \frac{1/2 w_H H_{F_{n-1}}}{\bar{w}_{F_n}},$$

$$Q_{F_n} = \frac{w_Q(Q_{F_{n-1}} + 1/4H_{F_{n-1}})}{\bar{w}_{F_n}}$$

$$\bar{w}_{F_n} = w_P(P_{F_{n-1}} + 1/4H_{F_{n-1}}) + 1/2 w_H H_{F_{n-1}} + w_Q(Q_{F_{n-1}} + 1/4H_{F_{n-1}})$$

where P_{F_n} is the genotypic frequency of the genotype pp in generation F_n; H_{F_n} is the genotypic frequency of the genotype pq in generation F_n; Q_{F_n} is the genotypic frequency of the genotype qq in generation F_n; F_n is the nth inbreeding generation; w_G is the fitness of the genotype G; and $\bar{w}_{F_n}$ is the fitness of the inbred population in generation F_n. These calculations were conducted using field emergence means, estimated across experiments, for populations BR-2 and SR-2.

Results

Segregation in F_4 populations

The 51 F_4 populations that were tested for segregation distortion of markers closely linked to *rhg1* varied in population size due to seed availability, field emergence, and missing marker data (Table 1). The observed segregation was significantly ($\alpha = 0.05$) distorted from the expected 7 RR:2 RS:7 SS ratio in 44 F_4 populations (86%) and the expected 1 RR:1 SS segregation ratio was distorted in 37 populations (73%). Of the 37 populations with significant distortion from the 1 RR:1 SS ratio, the observed number of plants in the RR marker class was fewer than expected in 33 populations and there were four populations with fewer plants than expected in the SS class. The chi-square association test indicated that the observed segregation ratios were not the same among the 33 F_4 populations with fewer than expected RR genotypes as well as among the four populations with fewer than expected SS genotypes ($P < 0.001$). The RR class was most severely underrepresented in the population developed from the cross LN94-480-97 × A94-774021 (0.13 RR:0.87 SS), and the SS class was most severely underrepresented in the population developed from 'Maverick' × A97-770012 (0.83 RR:0.17 SS).

Field emergence

Significant differences were detected in field emergence among NILs across locations in the 2002 experiments, and among NILs in the 2004 experiment ($P < 0.05$). The NIL by location interactions in the 2002 experiments were not significant at $\alpha = 0.25$, and their sums of squares were therefore pooled into the error term. In the ANOVA across the 2002 and 2004 experiments, close to significant effects among NILs were observed in BR-2 ($P = 0.076$) and significant NIL effects were detected in SR-2. For both populations, the NIL × year interaction was not significant at $\alpha = 0.25$ and the corresponding sums of squares were therefore pooled into the error term.

In both NIL populations grown in 2002, less field emergence was observed for SCN-resistant NILs compared to SCN-susceptible NILs (Table 2). In the experiment with BR-2, field emergence averaged 49.8% and SCN-resistant NILs had 5.7% less ($P < 0.05$) field emergence than susceptible NILs. In the SR-2 experiment, the average field emergence was 84.0% and resistant NILs had 2.7% less ($P = 0.079$) field emergence.

In the 2004 experiment, significant ($P < 0.05$) differences for the main effects of population, planting depth and SCN resistance, and a significant population by planting depth interaction were detected. A significant planting depth by NIL interaction at $\alpha = 0.25$ was not detected, and therefore the SCN resistance × planting depth interaction was not tested. In SR-2, field emergence was 75.4%, nearly twice as great as in BR-1 and BR-2. Across populations, seeds planted at a depth of 6 cm had 20.4% less field emergence than those planted at a depth of 3 cm. The effect of planting depth was significantly greater in BR-1 and BR-2 than in SR-2 explaining the significant population by planting depth interaction. Field emergence of SCN-resistant NILs was 4.4% less than emergence of SCN-susceptible NILs across populations and planting depths. Within populations, field emergence of SCN-resistant NILs was significantly less than susceptibles only in BR-1 (5.2%) and BR-2 (6.2%) averaged across planting depths. The difference in field emergence between resistant and susceptible NILs was 3.7% less in SR-2 than across BR-1 and BR-2. Across the 2 test years, resistant NILs had significantly less emergence than susceptible NILs in both populations. This difference was 5.9% in BR-2 and 2.5% in SR-2.

Table 1 Segregation ratios of DNA markers linked to the SCN-resistance locus *rhg1* in 51 F_4 populations segregating for SCN resistance

Parents		Year[a]		Marker segregation					Chi-square	
Resistant	Susceptible	Cross	Test	Marker	RR[b]	RS	SS	N[c]	7RR:2RS:7SS	1RR:1SS
A96-495005	Macon	1998	2000	Satt309	0.19	0.13	0.68	265	71.79***	72.04***
A97-973002	IA3010	1999	2001	Satt309	0.55	0.00	0.45	55	8.38*	0.45
A97-973002	LN97-338	1999	2001	Satt309	0.38	0.00	0.62	73	14.95***	3.96*
Dwight	A96-591046	1999	2001	Satt309	0.28	0.08	0.64	113	18.31***	15.38***
Dwight	LN97-16302	1999	2001	Satt309	0.44	0.04	0.52	235	18.10***	1.77
Dwight	LG94-1128	1999	2001	CTA	0.58	0.28	0.14	330	141.39***	87.97***
IA1008	A94-774021	1998	2000	Satt309	0.38	0.14	0.48	184	2.93	2.53
IA3005	A94-774021	1998	2000	Satt309	0.31	0.16	0.53	222	15.67***	13.44***
IA3005	LN91-1695	1998	2000	Satt309	0.23	0.08	0.70	394	107.00***	94.28***
Ina	A94-774021	1998	2000	Satt309	0.32	0.15	0.53	324	17.90***	16.75***
Ina	LN91-1695	1998	2000	Satt309	0.20	0.10	0.70	260	77.41***	73.03***
Ina	LN93-11945	1998	2000	Satt309	0.21	0.17	0.62	117	23.84***	22.77***
Ina	LN97-11702	1999	2001	CTA	0.37	0.00	0.63	244	51.20***	16.33***
Ina	LN97-16302	1999	2001	CTA	0.34	0.21	0.45	372	29.81***	6.00*
Ina	LN97-388	1999	2001	CTA	0.42	0.01	0.57	192	27.71***	4.13*
Ina	U97-3114	1999	2001	Satt309	0.41	0.19	0.40	328	12.42**	0.14
LN94-480-97	A94-774021	1998	2000	Satt309	0.11	0.16	0.74	102	46.84***	47.63***
LN94-480-97	LN91-1695	1998	2000	Satt309	0.30	0.18	0.53	331	28.11***	21.72***
LN95-15200-97	A94-774021	1998	2000	Satt309	0.43	0.12	0.46	276	0.47	0.26
LN95-15200-97	A96-494018	1999	2001	TMA5	0.16	0.09	0.74	183	71.89***	67.69***
LN95-15200-97	LN91-1695	1998	2000	Satt309	0.37	0.09	0.54	237	11.38**	8.17**
LN95-15200-97	LN97-16302	1999	2001	Satt309	0.27	0.22	0.51	384	54.96***	27.00***
LN95-15200-97	LN97-11702	1999	2001	Satt309	0.25	0.20	0.55	314	49.27***	35.96***
LN95-15200-97	LN97-388	1999	2001	Satt309	0.31	0.17	0.52	391	26.03***	20.19***
LN95-15200-97	Macon	1998	2000	Satt309	0.28	0.18	0.54	256	26.90***	20.22***
LN95-5454	A94-774021	1998	2000	Satt309	0.22	0.20	0.57	264	51.91***	40.30***
LN95-5454	LN91-1695	1998	2000	Satt309	0.40	0.12	0.48	222	1.79	1.65
LN95-5454	Macon	1998	2000	Satt309	0.29	0.14	0.57	356	31.98***	31.93***
LN95-6446	IA3010	1999	2001	Satt309	0.48	0.02	0.50	60	6.46*	0.02
LN97-26569	Macon	1999	2001	Satt309	0.34	0.12	0.54	374	16.36***	16.20***
LN97-26569	Savoy	1999	2001	CTA	0.40	0.18	0.41	371	11.55**	0.03
Loda	LG92-4208	1999	2001	Satt309	0.36	0.04	0.60	81	11.37**	5.13*
Loda	LG94-1128	1999	2001	CTA	0.28	0.03	0.69	180	48.60***	31.47***
Loda	LN97-16302	1999	2001	Satt309	0.36	0.17	0.47	168	5.94	2.6
Loda	Macon	1998	2000	Satt309	0.26	0.16	0.58	337	41.21***	38.96***
Loda	Olympus	1999	2001	Satt309	0.37	0.01	0.62	81	15.04***	5.00*
Loda	S22-C3	1999	2001	Satt309	0.32	0.05	0.63	57	9.23**	6.00*
LS93-0375	HS93-4118	1999	2001	Satt309	0.56	0.13	0.30	135	10.46**	10.47***
LS93-0375	Macon	1999	2001	Satt309	0.41	0.14	0.45	128	0.61	0.33
M90-184111	IA3010	1999	2001	Satt309	0.52	0.14	0.34	343	13.92**	13.45***
M90-184111	Macon	1999	2001	Satt309	0.44	0.17	0.38	344	9.01*	1.41
M90-184111	Savoy	1999	2001	CTA	0.37	0.13	0.50	365	7.26*	7.25**
Maverick	A96-591046	1999	2001	Satt309	0.48	0.03	0.49	77	6.92*	0.01
Maverick	A97-770012	1999	2001	Satt309	0.80	0.04	0.17	54	28.29***	22.23***
Maverick	LG92-1255	1998	2000	Satt309	0.22	0.11	0.67	280	65.62***	63.50***
Maverick	LN97-11702	1999	2001	Satt309	0.28	0.06	0.66	50	10.18**	7.68**
Maverick	LN97-16302	1999	2001	Satt309	0.34	0.03	0.64	77	14.76***	7.05**
Maverick	S21-A1	1999	2001	CTA	0.20	0.04	0.76	54	22.87***	17.31***
Pana	LG92-1255	1998	2000	Satt309	0.41	0.09	0.50	116	2.58	0.94
Pana	LN97-16302	1999	2001	Satt309	0.37	0.07	0.56	284	20.07***	10.60***
Rend	U97-3114	1999	2001	CTA	0.47	0.10	0.43	79	0.54	0.13
Across all populations [d]					0.35	0.11	0.54			

[a]Year of cross pollination and year of marker test
[b]*RR* genotypic frequency of F_4 plants homozygous for marker alleles associated with SCN resistance; *RS* genotypic frequency of F_4 plants heterozygous for marker alleles associated with SCN resistance; *SS* genotypic frequency of F_4 plants homozygous for marker alleles not associated with SCN resistance
[c]*N* population size
[d]The mean genotypic frequencies taken across populations, adjusted for differences in population size
*, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively

Effect of field emergence on segregation distortion

The calculations show that if greater field emergence is recessive rather than incompletely dominant or dominant, more severe segregation distortion during inbreeding will result, although these differences are not great (Table 3). Based on the germination results from BR-2 and assuming that greater emergence is recessive,

Table 2 Main effects, within-population effects, and interaction effects of several factors on field emergence (%) of three NIL soybean populations BR-1, BR-2, and SR-2 that were segregating for SCN resistance at the *rhg1* locus

Factor	Effect	Mean[a]	Contrast	
			Estimate	*P*
2002 experiments				
SCN resistance[b]	R vs. S in BR-2	49.8	−5.7	< 0.001
	R vs. S in SR-2	84.6	−2.7	0.079
2004 experiment				
Population	BR-1 vs. BR-2	39.4	−0.2	0.881
	BR-1 and BR-2 vs. SR-2	57.4	−36.0	< 0.001
Planting depth	3 vs. 6 cm	51.4	20.4	0.044
	3 vs. 6 cm in BR-1	39.3	25.9	< 0.001
	3 vs. 6 cm in BR-2	39.5	28.0	< 0.001
	3 vs. 6 cm in SR-2	75.4	7.3	0.108
SCN resistance	R vs. S	51.4	−4.4	< 0.001
	R vs. S in BR-1	39.3	−5.1	0.005
	R vs. S in BR-2	39.5	−6.2	< 0.001
	R vs. S in SR-2	75.4	−2.0	0.207
Population × SCN resistance	(R vs. S in BR-1) vs. (R vs. S in BR-2)	5.6	−1.1	0.613
	(R vs. S across BR-1 and BR-2) vs. (R vs. S in SR-2)	3.8	−3.7	0.057
Across experiments				
SCN resistance	R vs. S within BR-2	46.3	−5.9	< 0.001
	R vs. S within SR-2	81.6	−2.5	0.050

Field emergence was tested at two planting depths in the 2004 experiment
[a]Mean of the two contrast classes
[b]*R* NILs homozygous for the SCN-resistance allele at the *rhg1* locus; *S* NILs homozygous for the SCN-susceptibility allele at the *rhg1* locus

it was predicted that a population, which has undergone single-seed descent would have a segregation ratio of 0.38 RR:0.11 RS:0.52 SS in the F_4 generation and a ratio of 0.36 RR:0.02 RS:0.62 SS in the F_6 generation. Based on the SR-2 results and assuming that greater emergence is recessive, a ratio of 0.42 RR:0.12 RS:0.46 SS would be expected in the F_4 generation and of 0.45 RR:0.03 RS:0.52 SS in the F_6 generation.

Discussion

In the study with the F_4 populations, previous observations of fewer than expected plants or lines with resistance at *rhg1* were confirmed (Webb et al. 1995; Mudge et al. 1997; Prabhu et al. 1999; Glover et al. 2004). This suggests that segregation distortion at the *rhg1* locus is not caused by random drift but by selection, such as gametic selection, selection caused by seed abortion during development, and selection during seedling emergence and plant development. The association between *rhg1* resistance and segregation distortion suggests that the distortion is caused by pleiotropic effects of *rhg1* or other gene(s) linked with it.

It was shown that the distortion could be at least partially explained by differential emergence causing selection during population development. Based on

Table 3 Genotypic frequencies of the *rhg1* region during inbred line development depending on fitness (*w*)

Population		BR-2									SR-2								
Gene action		Recessive[a]			Incompletely dominant			Dominant			Recessive			Incompletely dominant			Dominant		
Genotypic class		RR[b]	RS	SS	RR	RS	SS	RR	RS	SS	RR	RS	SS	RR	RS	SS	RR	RS	SS
w		0.43	0.43	0.49	0.43	0.46	0.49	0.43	0.49	0.49	0.80	0.80	0.83	0.80	0.82	0.83	0.80	0.83	0.83
Inbreeding generation	F1	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00
	F2	0.24	0.48	0.27	0.23	0.50	0.27	0.23	0.52	0.26	0.25	0.50	0.26	0.25	0.50	0.25	0.24	0.50	0.25
	F3	0.34	0.23	0.43	0.34	0.25	0.41	0.33	0.27	0.40	0.37	0.25	0.39	0.37	0.25	0.38	0.36	0.25	0.38
	F4	0.38	0.11	0.52	0.37	0.12	0.51	0.36	0.14	0.49	0.42	0.12	0.46	0.42	0.12	0.45	0.42	0.13	0.45
	F5	0.38	0.05	0.57	0.37	0.06	0.57	0.37	0.07	0.56	0.45	0.06	0.49	0.45	0.06	0.49	0.44	0.07	0.49
	F6	0.36	0.02	0.62	0.36	0.03	0.61	0.36	0.04	0.60	0.45	0.03	0.52	0.45	0.03	0.52	0.45	0.03	0.51

Fitness was estimated from field emergence tests of each of two populations (BR-2 and SR-2) across three test locations
[a]Predictions calculated assuming greater emergence is recessive, incompletely dominant or dominant
[b]*RR* homozygous resistant; *RS* heterozygous; *SS* homozygous susceptible

emergence estimates in BR-2 and assuming that greater emergence is recessive, which is the scenario that causes the greatest segregation distortion, an F_4 segregation ratio of 0.38 RR:0.11 RS:0.52 SS was predicted. This is not greatly different than the average segregation ratio of 0.35 RR:0.11 RS:0.54 SS that was observed across all populations listed in Table 1.

Furthermore, the predicted segregation ratios are within the range of what has been observed by others. Glover et al. (2004) reported a segregation ratio of 0.32 RR:0.08 RS:0.59 SS in F_4 lines developed from the same cross as BR-1 and BR-2 (Bell × Colfax), while Mudge et al. (1997) observed a segregation ratio of 0.38 RR:0.62 SS in a $F_{4:5}$ population developed from a cross between PI 209332 and 'Evans'. Given the parameters listed previously, the segregation ratio in the F_5 generation was predicted to be 0.38 RR:0.05 RS:0.57 SS (or 0.40 RR:0.60 SS considering only the homozygous classes). This is similar to the segregation ratio of 0.38 RR:0.62 SS reported by Prabhu et al. (1999) for $F_{5:6}$ lines developed from a cross between Hartwig and 'Flyer', with Hartwig having resistance derived from Peking and PI 457654 (Anand 1992). The prediction for a segregation ratio in a F_6 generation of 0.36 RR:0.02 RS:0.62 SS (or 0.37 RR:0.63 SS considering only the homozygous classes) is only slightly more severe than the 0.38 RR:0.62 SS ratio observed by Webb et al. (1995) in $F_{6:7}$ lines derived from crossing the SCN resistance source PI 437654 with BSR101. The structure of the NIL populations does not allow one to test for the previously reported interaction between the *rhg1* locus and SCN resistance on LG M (Webb et al. 1995) or LG A2 (Prabhu et al. 1999).

The equations used to predict segregation distortion were derived for populations developed by single-seed descent. However, the F_4 populations tested in this study were derived by single-pod descent. Since an association between the *rhg1* resistance allele and lower yield were observed in Kopisch-Obuch et al. (2005), it is possible that the *rhg1* resistance allele is associated with smaller pod size through pleiotropy or genetic linkage. If such an association is present, pod size could be an additional distortion factor for the *rhg1* locus in the F_4 populations.

Although the F_4 results show an overall trend of more homozygous-susceptible plants than expected, segregation ratios vary widely among individual populations. This is obviously demonstrated by the 14 F_4 populations that were not distorted significantly and even more so by the four F_4 populations with a greater than expected number of homozygous-resistant plants. Even among populations with the same direction of segregation distortion, highly significant differences in segregation ratios were detected. An explanation for these differences among populations is that segregation observed in the study is conditioned by more than one distortion factor, possibly a cluster of several genes that might include pleiotropic effects of the SCN-resistance allele. In some of the resistant parents, it is possible that not all distortion factors are still linked with the *rhg1* marker. For this explanation to fit the observed results, however, each resistant parent should show a consistent degree of distortion in all crosses. This was not the general trend and for most resistant parents, there was even significant distortion in some crosses and no significant distortion in other crosses. Alternatively, there may be genes in some susceptible backgrounds that compensate for the distortion-causing factor in the resistant backgrounds. Unfortunately, there were not enough susceptible parents crossed with the same set of resistant parents to rigorously test this hypothesis. A susceptible parent that may have a compensatory effect is 'IA3010', which was crossed to three different resistant parents. In none of the populations derived from IA3010, there were significantly fewer than expected homozygous-resistant plants.

There was a trend of fewer homozygous-resistant plants in populations developed from crosses with Ina or LN95-15200-97, two parents that inherited resistance from PI 437654, Peking, and PI 88788, than in populations developed with resistance from PI 88788 or PI 209322. When distortion from 1 RR:1 SS is considered, 12 out of 14 (0.86) populations developed from crosses with Ina or LN95-15200-97 had significantly fewer RR than expected, whereas across populations developed from the other resistant parents, 25 out of 37 (0.68) had significantly fewer RR than expected. This trend is consistent with Brucker et al. (2005) who observed fewer than expected plants homozygous for *rhg1* resistance from PI 437654 in a population segregating for *rhg1* resistance alleles from both PI 88788 and PI437654.

While in the 2004 field emergence study plants were counted soon after emergence at VE to V2, in 2002 plant counts were taken at a later growth stage (V2 to V4), when selection for post-emergence plant vigor could have already had occurred. Such selection also could have caused segregation distortion and affected the results if associated with the *rhg1* locus. However, the consistency of the effects between 2002 and 2004, as indicated by the non-significant ($\alpha = 0.25$) NIL × year interaction leads one to conclude that if there were post-emergence stresses in 2002, these likely did not have a significant impact on the results. The only other resistance genes that have been mapped close to *rhg1* are two QTL that confer resistance to sudden death syndrome (SDS) caused by *Fusarium solani* (Mart.) Sacc. f. sp. *glycines* (Prabhu et al. 1999; Iqbal et al. 2001). Although there are *Fusarium* species that cause seedling blight and damping off, which could affect plant stands (Grau et al. 2005), there is no evidence that these QTL control resistance to seedling diseases.

The *rhg1* locus was associated with a significantly greater effect on field emergence in BR-1 and BR-2 than in SR-2. Interestingly, when Kopisch-Obuch et al. (2005) estimated the size of the segregating region flanking *rhg1* in all three NIL populations, they found that in the genotyped area the PI 88788 chromosomal region

flanking *rhg1* that was segregating in BR-1 and BR-2 was larger than the one segregating in SR-2. This supports the theory that the reduction in field emergence may be caused by more than one gene in the region surrounding *rhg1* possibly including pleiotropic effects of the resistance allele as already discussed in respect to the F_4 population results. In BR-1 and BR-2, Kopisch-Obuch et al. (2005) estimated that this segregating region was at least 9.1 cM in size, and potentially as large as 18.2 cM, whereas in SR-2 it was estimated to be at least 4.5 cM and as great as 9.2 cM in the genotyped area. Emergence affecting regions from PI 88788 that are segregating in BR-1 and BR-2, but not in SR-2, might therefore cause the greater suppression of field emergence associated with *rhg1* in the BR populations. However, the putative effect of the size and position of the segregating regions is confounded with a potential effect of the susceptible parent (Colfax in BR-1 and BR-2 versus S22-C3 in SR-2) and these results need to be confirmed in populations with the same genetic background.

The average field emergence in BR-1 and BR-2 was below 50% while it was above 80% in SR-2 (Table 2). Factors other than those linked to *rhg1* appear to be causing this poor emergence in BR-1 and BR-2, as the difference between the homozygous classes only accounts for a small amount of the low emergence in these populations. Hence, these populations are presumably fixed for one or more alleles that cause this overall low emergence rate. It is assumed that these alleles are mostly derived from Bell, since over years poor field emergence has been observed with this cultivar. As indicated by the significant effect of planting depth on field emergence observed in BR-1 and BR-2, at least part of the fixed low emergence alleles are presumably affecting seed vigor rather than seed germination per se.

Besides showing the effect of seedling emergence on segregation distortion, the results illustrate the challenges that breeders face when incorporating favorable genes from non-adapted exotic germplasm into elite breeding lines: the simultaneous introgression of unfavorable genes along with favorable genes from the donor genome due to linkage drag or pleiotropic associations with the target genes. The NIL populations used in the study revealed such an association between SCN resistance and unfavorable genes causing reduced field emergence. While soybean is able to compensate for lower plant density to a certain extent, due to its plasticity of growth, and thus avoid major yield loss, genetic associations with unfavorable traits might directly reduce yield. In such cases, extensive evaluation of the breeding material and the employment of selection strategies, such as marker-assisted breeding, are necessary to avoid yield depression when utilizing non-adapted germplasm in cultivar development.

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