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Bulked Segregant Analysis Using the GoldenGate Assay to Locate the *Rpp3* Locus that Confers Resistance to Soybean Rust in Soybean

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ABSTRACT

Few resistance loci to soybean rust (SBR), caused by *Phakopsora pachyrhizi* Syd., have been genetically mapped and linked to molecular markers that can be used for marker assisted selection. New technologies are available for single nucleotide polymorphism (SNP) genotyping that can be used to rapidly map traits controlled by single loci such as resistance to SBR. Our objective was to demonstrate that the high-throughput SNP genotyping method known as the GoldenGate assay can be used to perform bulked segregant analysis (BSA) to find candidate regions to facilitate efficient mapping of a dominant resistant locus to SBR designated *Rpp3*. We used a 1536 SNP GoldenGate assay to perform BSA followed by simple sequence repeat (SSR) mapping in an F₂ population segregating for SBR resistance conditioned by *Rpp3*. A 13-cM region on linkage group C2 was the only candidate region identified with BSA. Subsequent F₂ mapping placed *Rpp3* between SSR markers BARC_Satt460 and BARC_Sat_263 on linkage group C2 which is the same region identified by BSA. These results suggest that the GoldenGate assay was successful at implementing BSA, making it a powerful tool to quickly map qualitative traits since the GoldenGate assay is capable of screening 1536 SNPs on 192 DNA samples in three days.

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Abbreviations: BARC, Beltsville Agricultural Research Center; BSA, bulked segregant analysis; cM, centimorgan; LG, linkage group; LOD, likelihood of odds; nr, non-redundant; RB, dark reddish-brown lesions; SBR, soybean rust; SNP, single nucleotide polymorphism; SSR, simple sequence repeat; STS, sequence tagged site; TAN, tan lesions.

Soybean rust (SBR), caused by *Phakopsora pachyrhizi* Syd., was first discovered in North America in 2004 (Schneider et al., 2005) and has been detected in the United States as far north as Illinois and Indiana (Hartman et al., 2007; Mueller and Engelbrecht, 2007). Despite SBR not causing significant yield losses in North America, the potential of this pathogen to create epidemic outbreaks and to reduce soybean yields from 30 to 75% has been well documented in Brazil and Paraguay (Yorinori et al., 2005). The primary defense against this pathogen has been the widespread use of fungicides which can be very costly. The other defense currently available is host resistance, which has been found through germplasm screening.

The six known resistant sources (and their assigned locus names) for resistance to *P. pachyrhizi* (*Rpp*) come from the soybean

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accessions PI 200492 (*Rpp1*) (McLean and Byth, 1980), PI 230970 (*Rpp2*) (Hartwig and Bromfield, 1983), PI 462312 (*Rpp3*) (Hartwig and Bromfield, 1983), PI 459025 (*Rpp4*) (Hartwig, 1986), PI 200456 (*Rpp5*) (Garcia et al., 2008), and PI 506764 [*Rpp?*(Hyuuga)] (Monteros et al., 2007). Currently, five SBR resistance loci have been mapped on the soybean genetic linkage map. *Rpp1* maps to soybean linkage group (LG) G between SSR markers BARC_Sct_187 and BARC_Sat_064 (Hyten et al., 2007), *Rpp2* maps to LG J between SSR markers BARC_Sat_255 and BARC_Satt620 (Silva et al., 2008), *Rpp4* maps to LG G between SSR markers BARC_Satt288 and BARC_AF162283 (Silva et al., 2008), *Rpp5* maps to LG N between SSR markers BARC_Sat_275 and BARC_Sat_280 (Garcia et al., 2008), and *Rpp?*(Hyuuga) maps to LG C2 between SSR markers BARC_Satt460 and BARC_Satt134 (Monteros et al., 2007). Cultivar screening in Florida has found that the sources of *Rpp1*, *Rpp3*, and *Rpp?*(Hyuuga), along with several other germplasm accessions, show promising resistance to the *P. pachyrhizi* races that are currently in North America (D. Walker, personal communication, 2008). Currently, the map position of *Rpp3* is unknown. It is also unknown whether the other germplasm accessions that demonstrate resistance to the *P. pachyrhizi* races in North America contain one of the known rust resistance loci or a new resistant locus that can be deployed with the previously identified resistance loci. An efficient strategy of finding molecular markers associated with *Rpp3* along with quickly mapping resistance loci contained within new SBR resistant accessions is needed so that these resistance loci can be quickly integrated into breeding programs through marker-assisted selection and/or combined with other resistant loci. One strategy would be to combine the effectiveness of bulked segregant analysis (Michelmore et al., 1991) with a high-throughput genotyping method which is capable of screening many bulks with markers spread throughout the genome in a short period of time.

Single nucleotide polymorphisms (SNPs) are the most abundant genetic markers available in soybean (Choi et al., 2007; Hyten et al., 2006; Zhu et al., 2003). In addition, there have been myriad technologies developed to very quickly genotype large numbers of SNPs in DNA samples. The GoldenGate assay is a high-throughput SNP detection method, which is capable of screening 1536 SNP markers in three days on 192 DNA samples (Fan et al., 2003). In soybean, a 384 SNP GoldenGate assay was used to successfully map 345 SNPs onto the soybean consensus map and it was observed that the GoldenGate assay may be copy-number sensitive (Hyten et al., 2008). If the GoldenGate assay is copy-number sensitive it would be possible to score a bulk heterozygous despite not having equal amounts of the two alleles present, which would allow the assay to be effectively used for bulked segregant analysis. Our objective was to determine if the GoldenGate assay could be used for bulked segregant analysis to locate candidate region(s)

for *Rpp3* and then test the candidate regions(s) with SSR markers in a segregating population to determine the map location of *Rpp3* and to determine if BSA functioned successfully using the GoldenGate assay.

MATERIALS AND METHODS

Plant Material

PI 462312 was previously reported to carry the single dominant rust resistance locus *Rpp3* (Hartwig and Bromfield, 1983). A total of 110 F_2 -derived F_3 lines ($F_{2,3}$) from a cross between 'Williams 82' \times PI 462312 were used in this study. F_1 seeds were produced in the field at the Delta Research and Extension Center near Stoneville, MS during the summer of 2004. Seeds derived from individual F_1 and F_2 plants were produced during the winters of 2004–05 and 2005–06, respectively, at the USDA-ARS Tropical Agriculture Research Station near Isabela, PR. Each line consists of F_3 seeds derived from a single F_2 plant. Seeds of Williams 82, PI 462312, and PI 506764 (Hyuuga) were obtained from the USDA Soybean Germplasm Collection (USDA-ARS, Univ. of Illinois, Urbana, IL).

Soybean Rust Resistance Testing

All inoculations with *P. pachyrhizi* isolates (Table 1) were performed in the USDA-ARS Foreign Disease-Weed Science Research Unit Biosafety Level-3 Plant Pathogen Containment Facility at Ft. Detrick, MD (Melching et al., 1983) under the appropriate USDA Animal and Plant Health Inspection permit. There were two replications of the phenotyping of the *Rpp3* population that consisted of 110 F_2 -derived lines with five F_3 plants per line per replication. Two seeds per cell were planted in flats and thinned to a single plant per cell 10 d after planting as described by Hyten et al. (2007). Resistant and susceptible checks were planted randomly throughout the flats and included the resistant and susceptible parents, PI 462312 and Williams 82, respectively.

Inoculations were done on 15-d-old seedlings in sets of 10 to 22 flats each. Plants were inoculated with the *P. pachyrhizi* isolate IN73-1 as described by Hyten et al. (2007). The IN73-1 isolate produces dark reddish-brown (RB) lesions with few uredinia and some sporulation on accession PI 462312 and tan (TAN) lesions, which are due to many uredinia forming on the leaf and abundant sporulation, on Williams 82 (Hartwig and Bromfield, 1983). Resistant reactions were recorded when an RB lesion with few or no spores were observed on the unifoliolate or trifoliolate leaves (Hartwig and Bromfield, 1983). A susceptible TAN reaction was recorded when distinct tan lesions with prolific sporulation was observed on the unifoliolate or trifoliolate leaves (Bromfield and Hartwig, 1980).

In a second experiment in the USDA-ARS Foreign Disease-Weed Science Research Unit Biosafety Level-3 Plant Pathogen Containment Facility at Ft. Detrick, MD the soybean accession PI 506764, which has also been reported to be resistant to SBR (Monteros et al., 2007), was inoculated along with PI 462312 with 10 different *P. pachyrhizi* isolates (Table 1). There were two replications of the inoculations with two plants per line per replicate for each *P. pachyrhizi* isolate. Phenotyping was performed as previously described for the F_2 -derived population.

Bulked Segregant Analysis

Ten seeds each of PI 462312 and Williams 82 were grown and leaf tissue from the 10 plants was bulked and used for DNA extraction as described by Keim et al. (1988). Since *Rpp3* is a dominant resistance locus, three susceptible bulks were created for BSA to ensure that heterozygous *Rpp3* plants were not included in the bulks. A total of 26 $F_{2,3}$ lines gave a TAN reaction for all 10 of the F_3 plants tested. Three bulks of the homozygous susceptible lines were created. Two bulks consisted of nine $F_{2,3}$ lines and the third bulk was from leaf tissue of the remaining eight $F_{2,3}$ lines. DNA was extracted from the bulked leaf tissue of 10 F_3 plants from each $F_{2,3}$ line as described by Keim et al. (1988).

A total of 1536 SNP markers have been discovered and mapped onto the integrated molecular genetic linkage map using the GoldenGate assay (data not shown) as described by Hyten et al. (2008). These 1536 SNP markers were tested on PI 462312, Williams 82, and the three susceptible bulks using the GoldenGate assay and analyzed on the Illumina BeadStation 500G (Illumina, San Diego, CA) as described previously (Hyten et al., 2008). The automatic allele calling for each locus is accomplished with the GenCall software (Illumina, San Diego, CA). All GenCall data were manually checked, and positive hits for BSA were recorded when a SNP was polymorphic between Williams 82 and PI 462312 and all three susceptible bulks clustered tightly with Williams 82 in the GenCall output (Fig. 1).

Mapping of *Rpp3*

Before inoculation with *P. pachyrhizi* isolate IN73-1, a single leaflet was collected from the first trifoliolate or in some instances the whole second trifoliolate, from each of the 10 F_3 plants representing each of the 110 $F_{2,3}$ lines in the population screening described above. Leaf tissue was immediately frozen on dry ice. DNA was isolated from the leaf tissue using the Sigma REDExtract-N-Amp Plant PCR Kit (Sigma-Aldrich, St. Louis, MO) following the manufacturer's instructions. Beltsville Agricultural Research Center (BARC) SSR markers from the soybean consensus map (Choi et al., 2007) were tested within the candidate region identified in the GoldenGate assay to discover polymorphic SSR markers between Williams 82 and PI 462312. Polymorphic SSR markers in the candidate interval were used to screen six to 10 F_3 plants from each of the 110 $F_{2,3}$ lines. SSR genotyping was performed as described by Cregan et al. (1999). SSR allele size differences were determined as described by Wang et al. (2003) or with a 2% agarose gel. The genotype of each F_2 plant was inferred from the genotypes of its F_3 progeny. Map Manager QTX v. b20 (Manly et al., 2001) was used

Table 1. *Phakopsora pachyrhizi* isolates used in this study.

Isolate	Country	Location	Year collected	Source
AL04-1	United States	Mobile County, Alabama	2004	R. Frederick [†]
AU79-1	Australia	unknown	1979	unknown
BZ01-1	Brazil	Parana	2001	J. T. Yoriniori [‡]
HW94-1	United States	Oahu, Hawaii	1994	E. Kilgore [§]
IN73-1	India	Pantnagar	1973	D. N. Thapliyal [¶]
LA04-1	United States	Ben Hur, Louisiana	2004	R. Schneider [#]
PG01-2	Paraguay	Capitan Miranda	2001	W. M. Morel ^{††}
SA01-1	South Africa	Natal Province	2001	Z. A. Pretorius ^{‡‡}
TW72-1	Taiwan	Taipei	1972	L.-C. Wu ^{§§}
TW80-2	Taiwan	Taipei	1980	AVRDC ^{§§}

[†]Collections made with the assistance of T. Johnson, R. Wingard, and W. Harrison, Alabama Dep. of Agriculture and Industries, Montgomery, Alabama and E. Sikora, Alabama Cooperative Extension System, Auburn Univ., Auburn, Alabama.

[‡]Embrapa soja, Londrina, Brazil.

[§]Hawaii Department of Agriculture, Hilo, Hawaii.

[¶]Govind Ballabh Pant Univ. of Agriculture and Technology, Pantnagar, India.

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^{‡‡}Dep. of Plant Sciences, Univ. of the Free State, Bloemfontein, South Africa.

^{§§}Asian Vegetable Research and Development Center, Taipei, Taiwan.

with Kosambi's mapping function to estimate genetic distances between SSR markers and *Rpp3* in the 110 $F_{2,3}$ lines of Williams 82 × PI 462312. A minimum likelihood of odds (LOD) ≥ 3.0 and

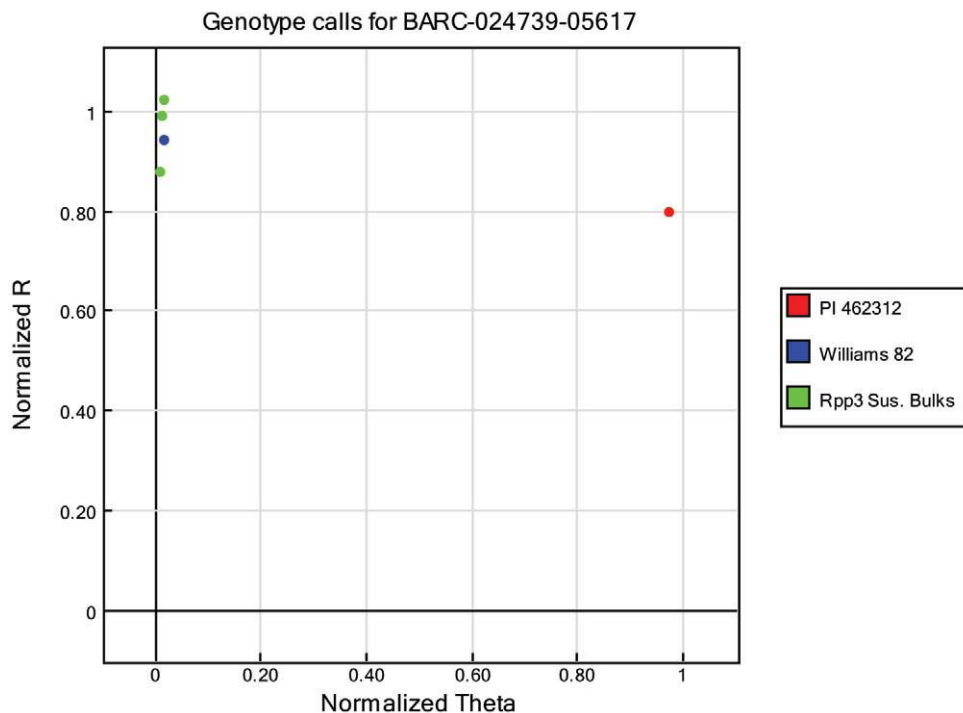


Figure 1. The clustering of a typical GoldenGate assay result that was considered a positive hit for bulked segregant analysis where the three susceptible bulks clustered with the susceptible genotype Williams 82. The normalized R (y axis) is the normalized sum of intensities of the two channels (Cy3 and Cy5) and normalized theta (x axis) is $[(2/\pi)\tan^{-1}(Cy5/Cy3)]$ where a normalized theta value nearest 0 is a homozygote for allele A and a theta value nearest 1 is homozygous for allele B (Fan et al., 2006).

a maximum distance of ≤ 50 centimorgan (cM) were used to test linkages among markers.

Molecular Characterization and Haplotyping of *Rpp3* Region

Once *Rpp3* was positioned between SSR markers on the soybean genetic map, the original sequence used to develop the SSR markers was compared to the 7 \times soybean genome sequence available at www.phytozome.net (Soybean Genome Project, DoE Joint Genome Institute) using BLASTN (Altschul et al., 1997). Scaffold 60 was identified to contain both flanking SSR markers. Annotation of the open reading frames for the region containing *Rpp3* were identified using a PARACEL BLASTX search using the NCBI non-redundant (nr) database with serial 20 kb genomic sequences starting at nucleotide 1,077,201 and continuing to nucleotide 1,977,200 in scaffold 60. The gene descriptions assigned to the BLASTX hits were compared to the preliminary annotation performed at www.phytozome.net, and discrepancies were manually inspected for accuracy.

A total of 48 primer pairs were designed using Primer3 (Rozen and Skaletsky, 2000) to scaffold 60 between nucleotides 1,077,201 and 1,977,200 (Supplemental Table 1). Primer pairs were checked using electronic PCR (Schuler, 1997) to verify that a single amplicon would be produced. Seven of the 48 were estimated to produce multiple amplicons in the soybean genome. The remaining 41 primer pairs were used to sequence Williams 82, PI 462312, and PI 506764. Additional haplotyping was performed on the soybean genotypes 'Archer,' 'Evans,' 'Minsoy,' 'Noir 1,' 'Peking,' and PI 209332. It has been demonstrated that these six genotypes discover 93% of the common SNPs (frequency > 10%) in a diverse *G. max* germplasm sample (Zhu et al., 2003). PCR amplification and sequencing reactions were performed as described by Choi et al. (2007). Sequencing was performed on the ABI 3730 DNA analyzer (Applied Biosystems, Foster City, CA) and SNP discovery performed as described by Matukumalli et al. (2006).

RESULTS

On the basis of the numbers of RB lesions (resistant) and TAN lesions (susceptible) among F₃ plants from each F_{2,3} line, the phenotype of each F₂ plant was inferred. As anticipated, the F₂ population fit a 3:1 (resistant:susceptible) ratio ($p = 0.74$). This segregation pattern agrees with the previous report that *Rpp3* is a single dominant resistance locus (Hartwig and Bromfield, 1983). The three bulks used for BSA were created from the 26 susceptible F_{2,3} lines with each bulk containing nine, nine, and eight different susceptible F_{2,3} lines.

A total of 27 of the 1536 SNPs screened with the GoldenGate assay were positive for BSA in all three susceptible bulks. A typical positive result for BSA in the GenCall software is shown in Fig. 1. A total of 1356 of the 1536 SNPs have been integrated into the previously published Choi et al. (2007) soybean consensus map (data not shown). The 27 SNPs that were positive for BSA were

all located within a 14 cM region on linkage group C2 between the SNP markers BARC-055889-13824 and BARC-053603-11920 (Supplemental Table 2).

Eight SSR markers around the candidate region for *Rpp3* were determined to be polymorphic between the mapping parents and were selected for genotyping in the F₂ population. While *Rpp3* is a dominant resistance locus, heterozygous F₂ plants were inferred from their F₂-derived F₃ progeny data which allowed *Rpp3* to be mapped as a codominant locus with the SSR markers. The resulting map placed *Rpp3* between SSR markers Satt460 and Sat_263 (Fig. 2). The map created by the F₂ population agrees well with the consensus map except for a map expansion between SSR markers Sat_263 and Satt316 (Fig. 2).

With knowledge of the map position of *Rpp3*, the sensitivity of the GoldenGate assay to the ratio of susceptible to resistant alleles in the bulked DNA samples could be investigated. Table 2 shows the number of Williams 82 and PI 462312 alleles at each of the SSR loci genotyped on one side of the *Rpp3* interval and the number of SNPs that clustered with the susceptible genotype within the intervals between the SSR loci. The first interval to contain SNPs that did not cluster with Williams 82 occurred in the interval between Satt489 and Satt365 in susceptible bulk #3. In this interval, the number of susceptible to resistant alleles was between 14:2 and 12:4. The next interval, between Satt365 and Sat_402, contained one SNP that did not cluster with Williams 82 in susceptible bulks #1 and #3. The number of alleles was 15:3 susceptible to resistant in bulk #1 and ranged from 12:4 to 10:6 in susceptible bulk #3 (Table 2).

The SBR resistance locus *Rpp3*?(Hyyuuga) in PI 506764 maps to the same region on LG C2 (Monteros et al., 2007) as *Rpp3*, indicating that they are the same locus with the same or different alleles or are two tightly linked loci. PI 462312 and PI 506764 were inoculated with 10 different foreign and domestic *P. pachyrhizi* isolates. The two accessions had identical rust reactions to all 10 isolates (Table 3). In addition to the isolate screening, the haplotypes of Williams 82, PI 462312, and PI 506764 were determined in the *Rpp3* region. On the basis of the sequence identity between SSR markers and the Soybean Genome Project, DoE Joint Genome Institute 7 \times soybean genome sequence, *Rpp3* is located on scaffold 60 (www.phytozome.net). Satt460 and Sat_263 are separated by a total of 897 kb of sequence. In this 897 kb of sequence, 31 PCR primer pairs spread an average of about 30 kb apart throughout this region produced a sequence tagged site (STS) for haplotype analysis of the three genotypes. A total of 292 SNPs were found in 25 STS while the other six STS were monomorphic. The positions of the 23 SNP-containing STS along with a gene annotation of the 897 kb region are shown in Fig. 3. A total of 275 of the 292 SNPs were successfully

Table 2. The number of Williams 82 and PI 462312 alleles at each of the SSR loci genotyped on one side of the interval containing *Rpp3* and the number of SNPs within the intervals between the SSR loci that clustered with the susceptible genotype (Williams 82) indicating a positive hit for bulked segregant analysis.

Susceptible bulk (no. susceptible families in bulk)	Number of alleles at Satt460†	Positive SNPs‡	Number of alleles at Sat_251†	Positive SNPs‡	Number of alleles at Satt489†	Positive SNPs‡	Number of alleles at Satt365†	Positive SNPs‡	Number of alleles at Sat_402†
1 (9)	18:0	11	18:0	4	18:0	6	15:3	1	15:3
2 (9)	18:0	11	18:0	4	18:0	6	18:0	2	16:2
3 (8)	16:0	11	15:1	4	14:2	3	12:4	1	10:6
Total # of informative SNPs§		11		4		6		2	

†Number of susceptible (Williams 82); resistant (PI 462312) alleles present within the bulk.

‡Number of SNPs within the SSR interval clustering with the susceptible parent, Williams 82.

§Number of SNPs within the SSR interval that were polymorphic between Williams 82 and PI 462312.

scored in both PI 462312 and PI 506764. Only two SNPs located approximately 67 kb away from Sat_263 differed between the two accessions (Supplemental Table 3).

DISCUSSION

The results of the genetic mapping, multiple isolate screening, and haplotyping of the resistance loci *Rpp3* and *Rpp2*(Hyuuga) from PI 462312 and PI 506764, respectively, strongly suggests that they are alleles of the same locus. Silva et al. (2008) reported that a *P. pachyrhizi* isolate collected from Brazilian fields is able to overcome the resistance found in PI 462312 while PI 506764 remains resistant. One explanation for this is that haplotyping only shows that *Rpp3* and *Rpp2*(Hyuuga) reside on the same ancestral haplotype and *Rpp3* and *Rpp2*(Hyuuga) could have diverged since the last common ancestor as evidenced by the two SNPs that are different within this interval between the two lines. Another plausible explanation is that the *P. pachyrhizi* isolate used by Silva et al. (2008) is a field population that has not been purified and could contain a mixture of heterogeneous isolates which could lead to a misclassification of susceptible TAN or resistant RB reactions. There could also be additional resistance loci that differ between the two accessions, which might account for differences in reaction phenotypes to this Brazilian field isolate of *P. pachyrhizi*. A complementation test is needed on a cross between PI 462312 and PI 506764 with an analysis of the progeny using a purified isolate of *P. pachyrhizi* that differentiates these two accessions to determine if *Rpp3* and *Rpp2*(Hyuuga) carry the same or different alleles for resistance.

The GoldenGate assay performed very well to define a putative genome position for the *Rpp3* locus using BSA. SSR data on the F_{2,3} lines that comprised each of the three susceptible bulks allowed the number of alleles contributed by the susceptible (Williams 82) vs. resistant (PI 462312) parent to be determined for each of the bulks. These data indicate that the GoldenGate assay is not completely sensitive to the presence of an alternative allele. A ratio of 7:1 (14 susceptible alleles to 2 resistant alleles) to 5:1 (15 susceptible

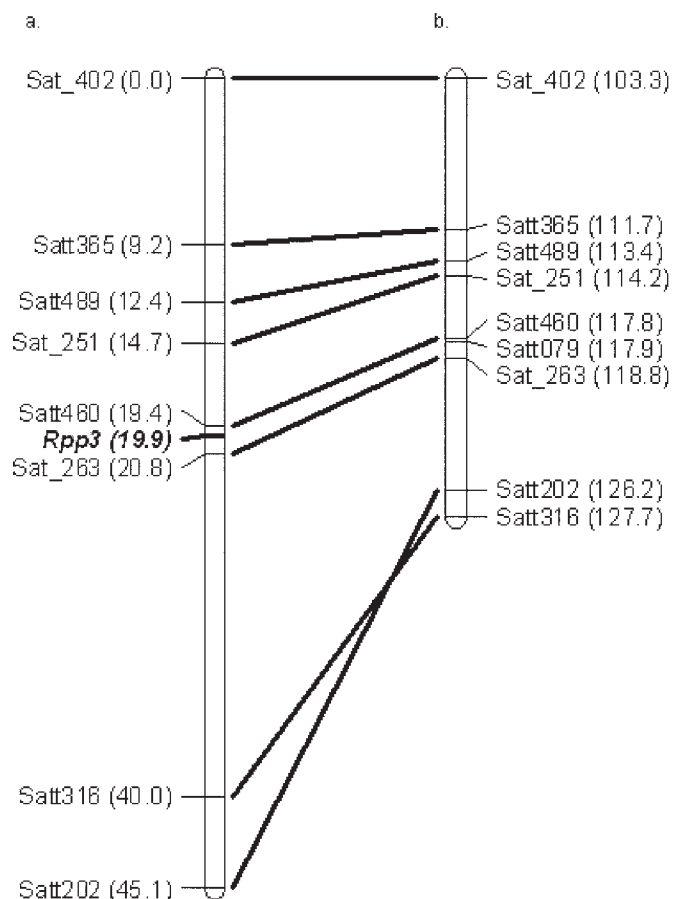


Figure 2. Genetic linkage maps of the *Rpp3* region of soybean linkage group C2. Cumulative cM distances are in parenthesis next to the marker name. The *Rpp3* resistance allele confers a reddish-brown lesion response to the *P. pachyrhizi* isolate IN73-1. a) Genetic map generated using the Kosambi's mapping function from 110 F_{2,3} lines of Williams 82 x PI 462312. b) Soybean consensus genetic map of the same SSR markers on linkage group C2 as reported by Choi et al. (2007).

alleles to 3 resistant alleles) susceptible to resistant alleles was enough to allow the detection of heterozygosity by some of the SNP assays, while heterozygosity was not detected by other SNP assays (Table 2). Despite the fact that some GoldenGate assays were not sufficiently sensitive to detect allele ratios of 5:1 in a heterozygous bulk, the use of three

Table 3. Soybean rust reaction of PI 462312, PI 506764, and Williams 82 screened in a biosafety level 3 plant pathogen containment facility with 10 different foreign and domestic *P. pachyrhizi* isolates.

ISOLATE	PI 462312 [†]	PI 506764	Williams 82
AL04-1	RB	RB	TAN
AU79-1	RB	RB	TAN
BZ01-1	RB/TAN	RB/TAN	TAN
HW94-1	RB	RB	TAN
IN73-1	RB	RB	TAN
LA04-1	RB	RB	TAN
PG01-2	RB/TAN	RB/TAN	TAN
SA 01-1	RB	RB	TAN
TW72-1	TAN	TAN	TAN
TW80-2	TAN	TAN	TAN

[†]RB = reddish-brown colored lesions, TAN = tan colored lesions, and RB/TAN = mostly reddish-brown colored lesions; a few tan colored lesions.

susceptible bulks eliminated all false positives and identified only one candidate region. This putative region was then confirmed to contain *Rpp3* through SSR mapping.

This study demonstrates that a 1536 GoldenGate reaction is an effective method for screening bulks created for traits controlled by a single locus. The GoldenGate assay is capable of screening 192 DNA samples in three days with 1536 SNPs. If three DNA bulks with their respective parents are used, 38 different bulk populations can be screened in three days. As more SBR resistance sources are identified and populations segregating for single loci are created, the GoldenGate assay will be an effective technique to rapidly determine if the resistance loci are located in a new genomic location or in a previously identified one.

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References

Altschul, S.F., T.L. Madden, A.A. Schaffer, J. Zhang, Z. Zhang, W. Miller, and D.J. Lipman. 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res.* 25:3389–3402.

Bromfield, K.R., and E.E. Hartwig. 1980. Resistance to soybean rust and mode of inheritance. *Crop Sci.* 20:254–255.

Choi, I.-Y., D.L. Hyten, L.K. Matukumalli, Q. Song, J.M. Chaky, C.V. Quigley, K. Chase, K.G. Lark, R.S. Reiter, M.-S. Yoon, E.-Y. Hwang, S.-I. Yi, N.D. Young, R.C. Shoemaker, C.P. Van Tassel, J.E. Specht, and P.B. Cregan. 2007. A soybean transcript map: Gene distribution, haplotype and single-nucleotide polymorphism analysis. *Genetics* 176:685–696.

Cregan, P.B., T. Jarvik, A.L. Bush, R.C. Shoemaker, K.G. Lark, A.L. Kahler, N. Kaya, T.T. VanToai, D.G. Lohnes, J. Chung, and J.E. Specht. 1999. An integrated genetic linkage map of the soybean genome. *Crop Sci.* 39:1464–1490.

Fan, J.B., K.L. Gunderson, M. Bibikova, J.M. Yeakley, J. Chen, E. Wickham Garcia, L.L. Lebruska, M. Laurent, R. Shen, and D. Barker. 2006. Illumina universal bead arrays. *Methods Enzymol.* 410:57–73.

Fan, J.B., A. Oliphant, R. Shen, B.G. Kermani, F. Garcia, K.L. Gunderson, M. Hansen, F. Steemers, S.L. Butler, P. Deloukas, L. Galver, S. Hunt, C. McBride, M. Bibikova, T. Rubano, J. Chen, E. Wickham, D. Doucet, W. Chang, D. Campbell, B. Zhang, S. Kruglyak, D. Bentley, J. Haas, P. Rigault, L. Zhou, J. Stuelpnagel, and M.S. Chee. 2003. Highly parallel SNP genotyping. *Cold Spring Harb. Symp. Quant. Biol.* 68:69–78.

Garcia, A., E.S. Calvo, R.A. de Souza Kiihl, A. Harada, D.M. Hiromoto, and L.G. Vieira. 2008. Molecular mapping of soybean rust (*Phakopsora pachyrhizi*) resistance genes: Discovery of a novel locus and alleles. *Theor. Appl. Genet.* 117:545–553.

Hartman, G.L., R.A. Hines, C.D. Faulkner, T.N. Lynch, and N. Pataky. 2007. Late season occurrence of soybean rust caused by *Phakopsora pachyrhizi* on soybean in Illinois. *Plant Dis.* 91:466.

Hartwig, E.E. 1986. Identification of a fourth major gene conferring resistance to soybean rust. *Crop Sci.* 26:1135–1136.

Hartwig, E.E., and K.R. Bromfield. 1983. Relationships among three genes conferring specific resistance to rust in soybeans. *Crop Sci.* 23:237–239.

Hyten, D.L., G.L. Hartman, R.L. Nelson, R.D. Frederick, V.C. Concibido, J.M. Narvel, and P.B. Cregan. 2007. Map location of the *Rpp1* locus that confers resistance to soybean rust in soybean. *Crop Sci.* 47:837–838.

Hyten, D.L., Q. Song, Y. Zhu, I.Y. Choi, R.L. Nelson, J.M. Costa, J.E. Specht, R.C. Shoemaker, and P.B. Cregan. 2006. Impacts of genetic bottlenecks on soybean genome diversity. *Proc. Natl. Acad. Sci. USA* 103:16666–16671.

Hyten, D.L., Q. Song, I.Y. Choi, M.S. Yoon, J.E. Specht, L.K. Matukumalli, R.L. Nelson, R.C. Shoemaker, N.D. Young, and P.B. Cregan. 2008. High-throughput genotyping with the GoldenGate assay in the complex genome of soybean. *Theor. Appl. Genet.* 116:945–952.

Keim, P., T.C. Olson, and R.C. Shoemaker. 1988. A rapid protocol for isolating soybean DNA. *Soybean Genet. Newsl.* 15:150–152.

Manly, K.F., R.H. Cudmore, Jr., and J.M. Meer. 2001. Map Manager QTX, cross-platform software for genetic mapping. *Mamm. Genome* 12:930–932.

Matukumalli, L.K., J.J. Grefenstette, D.L. Hyten, I.Y. Choi, P.B. Cregan, and C.P. Van Tassel. 2006. SNP-PHAGE-High throughput SNP discovery pipeline. *BMC Bioinformatics* 7:468.

McLean, R.J., and D.E. Byth. 1980. Inheritance of resistance to rust (*Phakopsora pachyrhizi*) in soybeans. *Aust. J. Agric. Res.* 31:951–956.

Melching, J.S., K.R. Bromfield, and C.H. Kingsolver. 1983. The plant pathogen containment facility at Frederick, Maryland. *Plant Dis.* 67:717–722.

Michelmore, R.W., I. Paran, and R.V. Kesseli. 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. *Proc. Natl. Acad. Sci. USA* 88:9828–9832.

Monteros, M.J., A.M. Missaoui, D.V. Phillips, D.R. Walker,

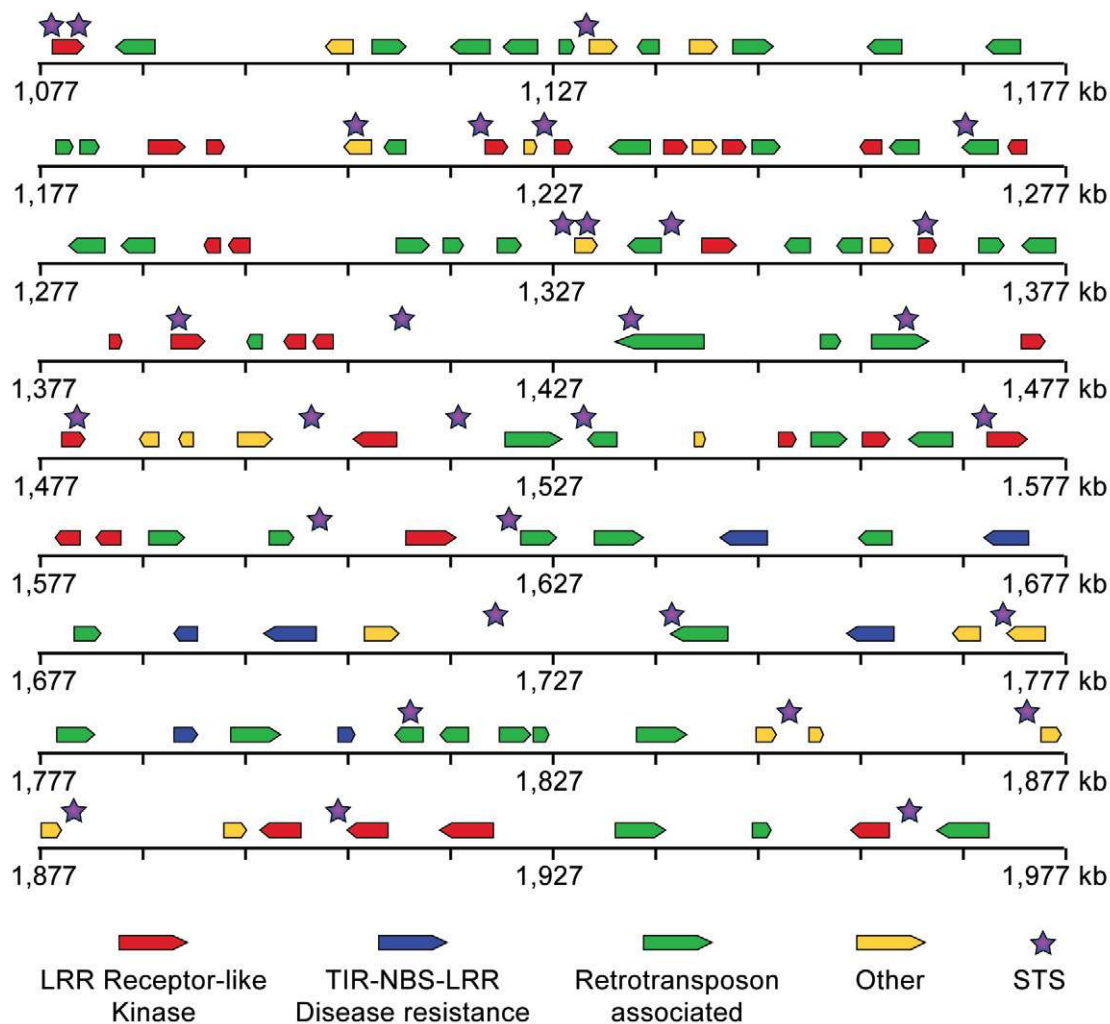


Figure 3. Diagram displaying the gene annotation for the region in the Soybean Genome Project, DoE Joint Genome Institute whole soybean 7x genome sequence scaffold-60 sequence between nucleotides 1,077,000 and 1,977,000. The SSR markers Satt460 and Sat_263 enclosing the *Rpp3* resistance locus are at either end of the genomic sequence depicted. BLASTX alignments with high sequence similarity to the NCBI non-redundant (nr) protein sequence database with an expected value < E-20 were marked and grouped based on similar protein descriptions. The group labeled LRR Receptor-like Kinase includes gene descriptions for S-locus, carbohydrate-binding, lectin, LRR transmembrane, and receptor-like kinases. The group labeled TIR-NBS-LRR was separated from the other LRR protein kinases because the sequence description for these proteins included the phrase “disease resistant LRR kinase.” The group labeled retrotransposon includes sequence descriptions of polyprotein, pol protein, retroelement, retrotransposon, retrotransposable, reverse transcriptase, retro-virus related, RT-like, integrase, RNA-directed DNA polymerase, transposase, and transposon. The STS group is the positions of sequence tagged sites used for haplotyping Williams 82, PI 462312, and PI 506764.

and H.R. Boerma. 2007. Mapping and confirmation of the ‘Huyuuga’ red-brown lesion resistance gene for Asian soybean rust. *Crop Sci.* 47:829–834.

Mueller, D., and C. Engelbrecht. 2007. Soybean rust found in Iowa [Online] http://www.ipm.iastate.edu/ipm/icm/2007/3-26/soybean_rust_found.html (posted 2007; verified 15 Dec. 2008).

Rozen, S., and H. Skaletsky. 2000. Primer3 on the WWW for general users and for biologist programmers. *Methods Mol. Biol.* 132:365–386.

Schneider, R.W., C.A. Hollier, H.K. Whitam, M.E. Palm, J.M. McKemy, J.R. Hernandez, L. Levy, and R. DeVries-Paterson. 2005. First report of soybean rust caused by *Phakopsora pachyrhizi* in the continental United States. *Plant Dis.* 89:774.

Schuler, G.D. 1997. Sequence mapping by electronic PCR. *Genome Res.* 7:541–550.

Silva, D.C., N. Yamanaka, R.L. Brogin, C.A. Arias, A.L. Nepomuceno, A.O. Di Mauro, S.S. Pereira, L.M. Nogueira, A.L. Passianotto, and R.V. Abdelnoor. 2008. Molecular mapping of two loci that confer resistance to Asian rust in soybean. *Theor. Appl. Genet.* 117:57–63.

Wang, D., J. Shi, S.R. Carlson, P.B. Cregan, R.W. Ward, and B.W. Diers. 2003. A low-cost, high-throughput polyacrylamide gel electrophoresis system for genotyping with microsatellite DNA markers. *Crop Sci.* 43:1828–1832.

Yorinori, J.T., W.M. Paiva, R.D. Frederick, L.M. Costamilan, P.F. Bertagnolli, G.E. Hartman, C.V. Godoy, and J. Nunes, Jr. 2005. Epidemics of soybean rust (*Phakopsora pachyrhizi*) in Brazil and Paraguay from 2001 to 2003. *Plant Dis.* 89:675–677.

Zhu, Y.L., Q.J. Song, D.L. Hyten, C.P. Van Tassell, L.K. Matukumalli, D.R. Grimm, S.M. Hyatt, E.W. Fickus, N.D. Young, and P.B. Cregan. 2003. Single-nucleotide polymorphisms in soybean. *Genetics* 163:1123–1134.