

Identification and molecular mapping of a major gene conferring resistance to *Phytophthora sansomeana* in soybean 'Colfax'

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Abstract

Phytophthora root rot (PRR) is one of the most important diseases in soybean (*Glycine max*). PRR is mainly caused by *Phytophthora sojae*, but recent studies showed that *P. sansomeana* is another causal agent of PRR with more aggressive symptoms, especially in seeds and seedlings. Unlike *P. sojae* which can be effectively managed by Rps genes, no known resistance genes have yet been reported for *P. sansomeana*. Our previous study screened 470 soybean germplasm for resistance to *P. sansomeana* and found that soybean 'Colfax' (PI 573008) carries major resistance to the pathogen. In this study, we crossed 'Colfax' with a susceptible parent 'Senaki' and developed three mapping populations with a total of 234 F₂:3 families. Inheritance pattern analysis indicated a 1:2:1 ratio for resistant: segregating: susceptible lines among all the three populations, indicating a single dominant gene conferring the resistance in 'Colfax' (designated Rpsan1). Linkage analysis using extreme phenotypes anchored Rpsan1 to a 30Mb region on chromosome 3. By selecting 9 polymorphic SNP markers within the region, Rpsan1 was genetically delimited into a 21.3 cM region between Gm03_4487138_A_C and Gm03_5451606_A_C, which corresponds to a 1.06 Mb genomic region containing 9 NBS-LRR genes based on Gmax2.0 assembly. The mapping results were then validated using two breeding populations derived from 'E12076T-03' × 'Colfax' and 'E16099' × 'Colfax'. Marker-assisted resistance spectrum analyses with 9 more isolates of *P. sansomeana* indicated that Rpsan1 carries a broad resistance to *P. sansomeana* isolates and has strong merit in protecting soybean from PRR in the future.

Key Message

The first single dominant resistance gene contributing major resistance to the oomycete pathogen *Phytophthora sansomeana* was identified and mapped from soybean 'Colfax'.

Introduction

Phytophthora root and stem rot (PRSR) is one of the most destructive diseases of soybean (*Glycine max*) in the U.S. It has been estimated that PRSR can cause an annual yield loss of more than 40 million bushels in the 28 soybean production U.S. states and Ontario, Canada (Allen et al. 2017). *Phytophthora sojae*, a soil-borne oomycete pathogen, has been considered the major causal agent of PRSR, yet other *Phytophthora* species, such as *P. sansomeana*, has attracted the attention of soybean community recently for its broad host range, wide distribution and high pathogenicity, and has becoming emerging threat for soybean production (Hansen et al 2009; Hansen et al 2012; Rojas et al 2017; Detranaltes et al. 2022; Hebb et al. 2023a).

P. sansomeana causes similar symptoms to *P. sojae*, such as seed and root rot (Dorrance et al. 2018). However, unlike *P. sojae* which primarily hosts soybean, *P. sansomeana* infects more plant species, such as corn (*Zea mays* L.), Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), white clover (*Trifolium repens* L.), and several weed species such as wild carrot (*Daucus carota* L.) (Hacker et al 2005, Hansen et al 2009; Zelaya-Molina et al 2010; Rahman et al 2015; Rojas et al 2017; Chang et al 2017; McCoy et al 2018;

An et al 2019; Rojas et al 2019). *P. sansomeana* has been identified in Indiana, Ohio, Illinois, Michigan, South Dakota in the U.S., and other countries such as Canada, Iran, Japan, and China (Reeser et al 1991; Malvick and Grunden 2004; Zelaya-Molina et al 2009; Bienapfl et al 2011; Rahman et al 2015; Rojas et al 2017; Safaiefarahani et al 2016; Rojas et al 2019; Tande et al 2020; Tang et al 2010). An in-vitro comparative pathogenicity study indicated that *P. sansomeana* was more aggressive than *P. sojae* using measures of root area, root length, and dry weight (Rojas et al 2017).

The most effective and economical way to manage PRSR is the deployment of resistance genes (Dorrance et al. 2004, Lin et al. 2013). For *P. sojae*, more than 40 *Rps* (Resistance to *Phytophthora sojae*) genes/alleles have so far been identified distributing on 9 soybean chromosomes. Intriguingly, more than half of them were mapped between 2.9Mb and 7.6Mb (*Gmax2.0*) on the short arm of chromosome 3, including *Rps1k* (Lin et al. 2022). *Rps1k* has been widely deployed in soybean varieties for protection against *P. sojae* in the past decades and consists of four coiled-coil (CC) nucleotide binding site-leucine rich repeat (CC-NBS-LRR) type of resistance genes (Bhattacharyya et al. 2005). For *P. sansomeana*, two minor effect quantitative resistance loci (*qPsan5.1* and *qPsan16.1*) has been reported on Chr. 5 and Chr. 6, respectively, with epistatic interactions (Lin et al. 2021). However, little is known if any resistance genes exist in soybean plants, and none of them has been identified.

In our previous study, we screened 470 soybean germplasm for their resistance against *P. sansomeana* using modified hypocotyl inoculation method (Salman et al. submitted), and identified that one of the lines, 'Colfax' (PI 573008), carries major resistance to *P. sansomeana* isolates. Therefore, the objectives of this study were to 1) dissect the inheritance pattern of resistance using segregation populations derived from 'Colfax' and a susceptible line 'Senaki', 2) determine the genetic location of the resistance gene on soybean chromosomes using molecular markers, 3) validate the linkage of markers using two breeding populations, and 4) characterize the resistance spectrum of the resistance gene against more *P. sansomeana* isolates.

Materials and Methods

Plant materials

Three F2:3 mapping populations (designated CS3, CS5, and CS6, respectively) were developed by crossing the resistant parent 'Colfax' (PI 573008) with the susceptible parent 'Senaki' (PI 507699) (Colfax × Senaki) in 2018. The F1 seeds were self-pollinated to produce F2 populations in 2019 and advanced to F2:3 families in 2020 at MSU agronomy farm. 96, 107, and 31 F2:3 families were obtained for CS3, CS5, and CS6, respectively, making a total of 234 segregating F2:3 families.

Two breeding populations (POP200032 and POP200040) were used as validation populations which were developed by crossing 'Colfax' (used as) with 'E12076T-03' (POP200032) and 'E16099' (POP200040), respectively, in 2020. E12076T-03 and E16099 are improved soybean varieties with high protein and high yield potential developed by MSU soybean breeding program and are both susceptible to

P. sansomeana. The F1s of the crosses were self-pollinated in 2021 and advanced to F3:4 families using single seed decent (SSD) method in the greenhouse in 2022. To validate the genetic position of the resistance gene, 82 F3:4 families from POP200032 and 150 F3:4 families from POP200042 were used.

Isolates of *P. sansomeana*

For genetic mapping and genetic validation, *P. sansomeana* isolate *MPS17-22* (McCoy et al. 2022) was used to challenge the three CS populations and the two validation populations. In addition, nine *P. sansomeana* isolates (*C-KSSO2 3-6*, *VAL 1602-84*, *C-NESO2 5-12*, *KSSO 6 - 1*, *MICO3-24*, *VAL 1602-18*, *MPS17-24*, *V-KSSO2 3-6*, and *C-ISASO2 6-15*) were used to investigate the resistance spectrum of the resistance gene. The above isolates were selected based on their diversity in aggressiveness, host range, and geographical distributions to best represent the screened *P. sansomeana* isolates (Rojas et al. 2017; McCoy et al. 2022). All the isolates were stored long term on potato carrot agar slants and hemp seed vials. *P. sansomeana* inoculum was prepared by transferring a 5-mm agar plug from an actively growing isolate to 100mm × 15mm petri dish plates containing dilute lima bean agar (LBA) (Dorrance et al 2008). The plates were then incubated at room temperature (22–24°C) for 10–14 days before use.

Disease evaluations and inheritance pattern analysis

A modified hypocotyl inoculation method was used to test the response of soybean lines to *P. sansomeana* (Dorrance et al. 2004; Lin et al. 2013). In this method, 7 to 10 days old seedlings (12 seeds/replicate) were inoculated with the pathogen colonies grown on lima bean agar medium (Dorrance et al., 2008). The inoculum was chopped into 2mm × 4mm pieces and inserted into the hypocotyl of soybean seedlings using a sharp spatula. Inoculated pots were then covered with a humidity dome and placed at room temperature (20 to 24°C) and a 12-hour photoperiod was provided. Twenty-four hours after inoculation, the humidity dome was removed, and the seedlings were kept growing in the same environment until seven days post inoculation (dpi) when all the seedlings were subject to disease evaluation. The phenotype of an F2:3 family was evaluated using the ratio of resistant progenies (survival with no symptoms). An F2:3 family was considered homozygous resistant if more than 70% of the progenies survived, segregating if 30–70% survived and susceptible if less than 30% survived.

A Chi-square test was used to test the phenotypic data for a goodness-of-fit to the ratio of 1:2:1 for the resistance to 234 F2:3 families against *P. sansomeana* isolate *MPS17-22*. All the statistical calculations were performed using IBM SPSS Statistics Premium 29 software.

Sample collection and DNA extraction

Leaf samples of 12 F3 seedling plants were bulk collected to represent the genotype of each F2 line. Genomic DNA of the bulk samples was extracted using a standard Cetyl Trimethyl Ammonium Bromide (CTAB) method and the DNA pellet was dissolved in 200µl 10mM Tris-HCl buffer. DNA samples were quantified using an ND-1000 Spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA).

Linkage analysis of extreme phenotypes

Forty-five most resistant F2:3 families and 44 most susceptible F2:3 families were used for linkage analysis. The 89 families were genotyped using Illumina Infinium BARCSoySNP6K iSelect BeadChip genotyping array (Illumina, San Diego, USA) (Song et al. 2013). The genotype data of the parental lines Colfax (PI 573008) and Senaki (PI 507699) were obtained from SoySNP50K database in soybase (www.soybase.org), and subsets of 6K data of the two parental lines were extracted, from which 1,599 polymorphic SNP markers were identified.

Genetic mapping

Extreme phenotype analysis indicated a 10Mb genomic region on chromosome 3 that was associated with the *P. sansomeana* resistance in Colfax. To further delimit the genomic interval of the resistance gene, nine additional polymorphic SNP markers spanning the associated region were selected from the SoySNP50K database for KASP (Kompetitive Allele-Specific PCR) assay analysis (LGC Biosearch Technologies). The nine markers (Gm03_3391237_A_G, Gm03_3828735_G_A, Gm03_4487138_A_C, Gm03_5451606_A_C, Gm03_5808835_C_T, Gm03_6844115_A_C, Gm_8205334_A_G, Gm03_14228358_T_C, and Gm03_22189671_C_T) were then used to genotype all the F2:3 families to map the resistance gene. Linkage maps were constructed using MAPMAKER/EXP Version 3.0 (Lander et al. 1987), and linkage groups were determined using a logarithm of the odds (LOD) score of 3.0b, with Kosambi's mapping function deployed.

Marker-assisted resistance spectrum (MARS) analyses

To characterize the resistance spectrum of *Rpsan1*, the flanking markers of *Rpsan1* (Gm03_4487138_A_C and Gm03_5451606_A_C) were used and identified 15 homozygous resistance genotypes (*Rpsan1/Rpsan1*, R group) and 15 homozygous susceptible genotypes (*rpsan1/rpsan1*, S group) from the CS mapping populations. These selected F2:3 families were then challenged against 9 additional *P.sansomeana* isolates using the hypocotyl inoculation method. Due to the availability of seeds, for each F2:3 family, one replicate with 12 seeds was used for the test, and the average of all the F2:3 families of the same genotype was considered the overall performance of the genotype.

Results

Inheritance pattern of resistance to *P. sansomeana*

The three F2:3 mapping populations, CS3, CS5, and CS6, were challenged with *P. sansomeana* isolate *MPS17-22* for inheritance pattern analysis. Of the 96 F2:3 families of CS3 population, 16 were identified as homozygous resistant, 51 were identified as segregating, and 29 were identified as susceptible. The observed number of 16:51:29 fits the 1:2:1 ratio very well ($\chi^2_{1:2:1} = 23.896$, $p = 0.143$) using goodness-of-fit test. Similarly, the 107 F2:3 families of CS5 and 31 F2:3 families of CS6 both fit the 1:2:1 ratio as well, with $\chi^2_{1:2:1}$ of 2.495 and 1.903, and p value of 0.287 and 0.386, respectively. Combined analysis of all the 234 F2:3 families of the three mapping populations yielded an observed number of 58:121:55 for homozygous resistance: segregation: susceptible, which fit the ratio of 1:2:1 perfectly ($\chi^2_{1:2:1} = 0.350$, $p =$

0.839) (Table 1). All these results indicated that a single dominant gene confers the resistance against *P. sansomeana*, and this gene is designated *Rpsan1* (for Resistance to *P. sansomeana*).

Table 1
Inheritance pattern of the F2:3 families (Colfax × Senaki) to *P.sansomeana* isolate MPS17-22 using goodness-of-fit test

F2:3 Populations	Population size	Observed (Res:Seg:Sus)	Expected (Res:Seg:Sus)	$\chi^2_{1:2:1}$	P
CS3	96	16:51:29	24:48:24	3.896	0.143
CS5	107	31:56:20	26.75:53.5:26.75	2.495	0.287
CS6	31	11:14:6	7.75:15.5:7.75	1.903	0.386
Combined	234	58:121:55	58.5:117:58.5	0.350	0.839
Res: homozygous resistant. Seg: segregation. Sus: susceptible.					

Table 2
Marker assisted resistance spectrum (MARS) analyses of *Rpsan1* against additional *P. sansomeana* isolates using 30 F2:3 families from the mapping populations.

<i>P. sansomeana</i> isolate	F2:3 families selected		Colfax	Senaki	Williams82
	R group (Mean ± SE)	S group (Mean ± SE)			
<i>C-KSSO2 3-6</i>	0.29 ± 0.05 ^{a*}	0.11 ± 0.05 ^b	0.27 ± 0.20 ^{ab}	0.06 ± 0.03 ^{ab}	0.00 ± 0.00 ^b
<i>VAL160 2-84</i>	0.30 ± 0.06 ^a	0.05 ± 0.02 ^b	0.37 ± 0.08 ^a	0.13 ± 0.05 ^b	0.02 ± 0.03 ^b
<i>CNESO2 5-12</i>	0.48 ± 0.07 ^a	0.02 ± 0.01 ^b	0.51 ± 0.09 ^a	0.14 ± 0.06 ^b	0.00 ± 0.00 ^b
<i>KSSO 6-1</i>	0.44 ± 0.07 ^a	0.07 ± 0.02 ^b	0.38 ± 0.05 ^{ac}	0.18 ± 0.04 ^{bc}	0.00 ± 0.00 ^b
<i>MICO3-24</i>	0.45 ± 0.09 ^a	0.07 ± 0.04 ^b	0.36 ± 0.09 ^a	0.07 ± 0.04 ^b	0.00 ± 0.00 ^b
<i>VAL160 2-18</i>	0.84 ± 0.04 ^a	0.31 ± 0.05 ^b	0.81 ± 0.05 ^a	0.21 ± 0.11 ^b	0.12 ± 0.02 ^b
<i>MPS17-24</i>	0.44 ± 0.08 ^a	0.08 ± 0.03 ^b	0.77 ± 0.07 ^c	0.38 ± 0.08 ^a	0.06 ± 0.06 ^b
<i>V-KSSO2 3-6</i>	0.90 ± 0.03 ^a	0.73 ± 0.07 ^b	1.00 ± 0.00 ^a	0.78 ± 0.15 ^{ab}	0.47 ± 0.06 ^c
<i>C-IASO2 6-15</i>	0.62 ± 0.07 ^a	0.18 ± 0.04 ^b	0.65 ± 0.15 ^a	0.53 ± 0.03 ^a	0.00 ± 0.00 ^b
*: Different letters indicate significant difference between groups at 0.05 level using LSD.					

Linkage analysis of *Rpsan1* using extreme phenotypes

Considering that it is more difficult to distinguish the homozygous resistant and heterozygous resistant phenotypes, we used the extreme susceptible phenotypes to start our linkage analysis. Of the 44 extreme susceptible lines, the percentage of 'Senaki' genotype (represented by 'b') was calculated for all the 1,599 polymorphic SNP markers across 20 chromosomes (Supplementary Fig. 1). Ideally, if a marker is co-segregating with *Rpsan1*, all the 44 extreme susceptible lines will be expected to be 'b' genotype. In our results, we found that the highest percentage of genotype 'b' is the marker Gm03_5733321_A_G, with 84% of 'b' genotype on chromosome 3. Further investigation of this region uncovered a continuous genomic region of ~32Mb with more than 60% 'b' genotypes (Fig. 1) between SNP markers Gm03_2224975_G_A and Gm03_35797299_A_G. Moreover, this region can be validated from the percentage of 'a' genotype in the 45 extreme resistance lines, with an average of 41%, compared to the average of 26% across the whole genome (Fig. 1). Therefore, *Rpsan1* is very likely located within the 32Mb genomic region on soybean chromosome 3.

Genetic mapping of *Rpsan1* using 234 F2:3 families

To further delimit the genetic position of *Rpsan1*, 9 polymorphic SNP markers from the 6k chip were selected spanning the 32Mb genomic regions for genetic mapping using all the 234 F2:3 families. The 9 markers covered a genetic region of 99.1cM, and the order of the markers were in good agreement with Williams82 reference genome on chromosome 3 (MLG N) (Schmutz et al. 2010; Song et al. 2013). *Rpsan1* was mapped between Gm03_4487138_A_C and Gm03_5451606_A_C, with a genetic distance of 10.1cM and 11.2cM, respectively (Fig. 2). The mapped region of *Rpsan1* corresponded to a physical distance of 1.06 Mb on chromosome 3 according to Gmax2.0 assembly, which contains 9 NBS-LRR type of genes (www.soybase.org).

Validation of the *Rpsan1* locus in two breeding populations

Two polymorphic markers, Gm03_5808835_C_T and Gm03_8205334_A_G, were identified and used for POP200032, from which 19 homozygous resistant (*Rpsan1/Rpsan1*) genotypes (R1 group) and 19 homozygous susceptible (*rpsan1/rpsan1*) genotypes (S1 group) were identified. Gm03_4487138_A_C and Gm03_5808835_C_T were found polymorphic between E16077 and Colfax and were therefore used for POP200040. Using the two markers, 16 F2:3 families were identified as '*Rpsan1/Rpsan1*' genotypes (R2 group) and 21 F2:3 families were identified as '*rpsan1/rpsan1*' genotypes (S2 group) in POP200040. All the selected lines were inoculated with *P. sansomeana* isolate *MPS17-22*. As expected, the average ratio of resistant progenies in R1 group is 0.685, which is close to that of Colfax (0.734), but significantly higher than that of S1 group (0.132) and E12076T-03 (0.067) (Fig. 3A). For 200040, the average ratio of resistant progenies in R2 group is 0.714, which is significantly higher than that of S2 (0.193) and E16077 (0.000) (Fig. 3B). Therefore, the testing results from POP200032 and POP200040 confirmed that the markers are tightly linked with the *Rpsan1* locus.

Resistance spectrum of *Rpsan1* to additional *P. sansomeana* isolates

Of the 9 isolates of *P. sansomeana* tested in this study, the parental line 'Colfax' showed intermediate to complete resistance to all of them. The other parent, 'Senaki', was susceptible to six of the isolates, but appeared intermediate resistant or resistant to three isolates (*MPS17-24*, *V-KSSO2 3-6*, and *C-IASO2 6-15*). As a control, 'Williams82' was susceptible to 8 of the 9 isolates but appeared mediate resistant to *V-KSSO2 3-6*. For the selected genotype groups, the R group (*Rpsan1/Rpsan1*) showed intermediate or complete level of resistance to all the 9 isolates and was consistent with the reaction of 'Colfax'. The S group (*rpsan1/rpsan1*) was susceptible to 8 of the 9 isolates but resistant to *V-KSSO2 3-6*, which is consistent with the response of 'Senaki'. All these results confirmed that *Rpsan1* is the only gene in 'Colfax' conferring resistant to all the *P. sansomeana* isolates.

Discussion

In this study, the first *P. sansomeana* resistance gene, *Rpsan1*, was identified from a soybean line 'Colfax'. *Rpsan1* was mapped in a 1.06Mb genomic region between Gm03_4487138_A_C and Gm03_5451606_A_C on soybean chromosome 3, which harbors 9 NBS-LRR type of genes. Interestingly, this region is also a hot-spot of more than 20 *Rps* genes/alleles conferring resistance to *P. sojae* (Lin et al. 2022), of which *Rps1k* has been shown to contain four CC-NBS-LRR genes (Bhattacharyya et al. 2005). It's therefore likely that the *Rpsan1* gene is an NBS-LRR type of resistance gene. Further fine mapping for map-based cloning of *Rpsan1* region using large segregation populations may be needed.

It has been well-documented that the interaction of *Rps* genes and *P. sojae* follows a gene-for-gene pattern, where an *Rps* gene may recognize a specific avirulence (*Avr*) factors secreted by the pathogen and trigger hypersensitive response (Dorrance et al. 2018). More than 11 *Avr* genes have been identified from the *P. sojae* genome, all of which encode a RxLR (arginine-anyamino acid-leucine-arginine) motif (Anderson et al. 2015). These RxLR motif containing proteins play a critical role in gene-for-gene resistance against *Phytophthora* species, through recognition by the host resistance genes (Anderson et al. 2015). Intriguingly, through whole genome sequencing and gene annotation of two isolates of *P. sansomeana*, 165 and 155 candidate RxLR effector genes were predicted from the high pathogenicity isolate (*Psan_HP*) and the moderately pathogenic isolate (*Psan_MP*), respectively (Hebb et al. 2023b). Therefore, it may not be too surprising to identify more resistance genes in soybean, which may interact with the *Avr* effectors from *P. sansomeana* for a gene-for-gene resistance.

Marker-assisted selection (MAS) has been proved with high efficiency and accuracy in selecting desirable alleles and has been widely deployed in plant breeding programs (Ribaut and Hoisington 1998). In this study, we applied the flanking markers of *Rpsan1* in two breeding populations and showed that the markers can be efficiently used for selecting resistant genotypes. However, the flanking markers are not perfect for MAS because they are still at least 10.1cM (corresponding to a 1.06Mb genomic distance) away from the *Rpsan1* locus (Fig. 2). To identify perfect markers for MAS, a large segregating population

may be needed to delimit the marker towards pinpointing the gene. For example, 17,050 segregating F4 plants were used to fine map *Rps11* for *P. sojae* resistance (Wang et al. 2021). Using a map-based cloning approach, the *Rps11* locus was finally delimited to a 151kb region, and the 27.7kb NBS-LRR type of candidate gene was proved to be *Rps11* through expression and transformation methods. In this study, we have started screening more than 2,000 F3:4 segregating populations and perfect markers for MAS are expected to be identified in the near future.

Declarations

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Author Contribution Statement

DW and FL designed the research. FL, MS, ZZ, AGM, WL, RTM, DM, MX, and CG carried out the experiments. FL and MS analyzed the data and developed the draft manuscript. AGM, MIC, and DW edited the manuscript. All authors revised the manuscript and contributed to the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Data availability

The phenotype and genotype data generated in this study is available in 'Supplementary Data'. Additional information is available upon request.

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Figures

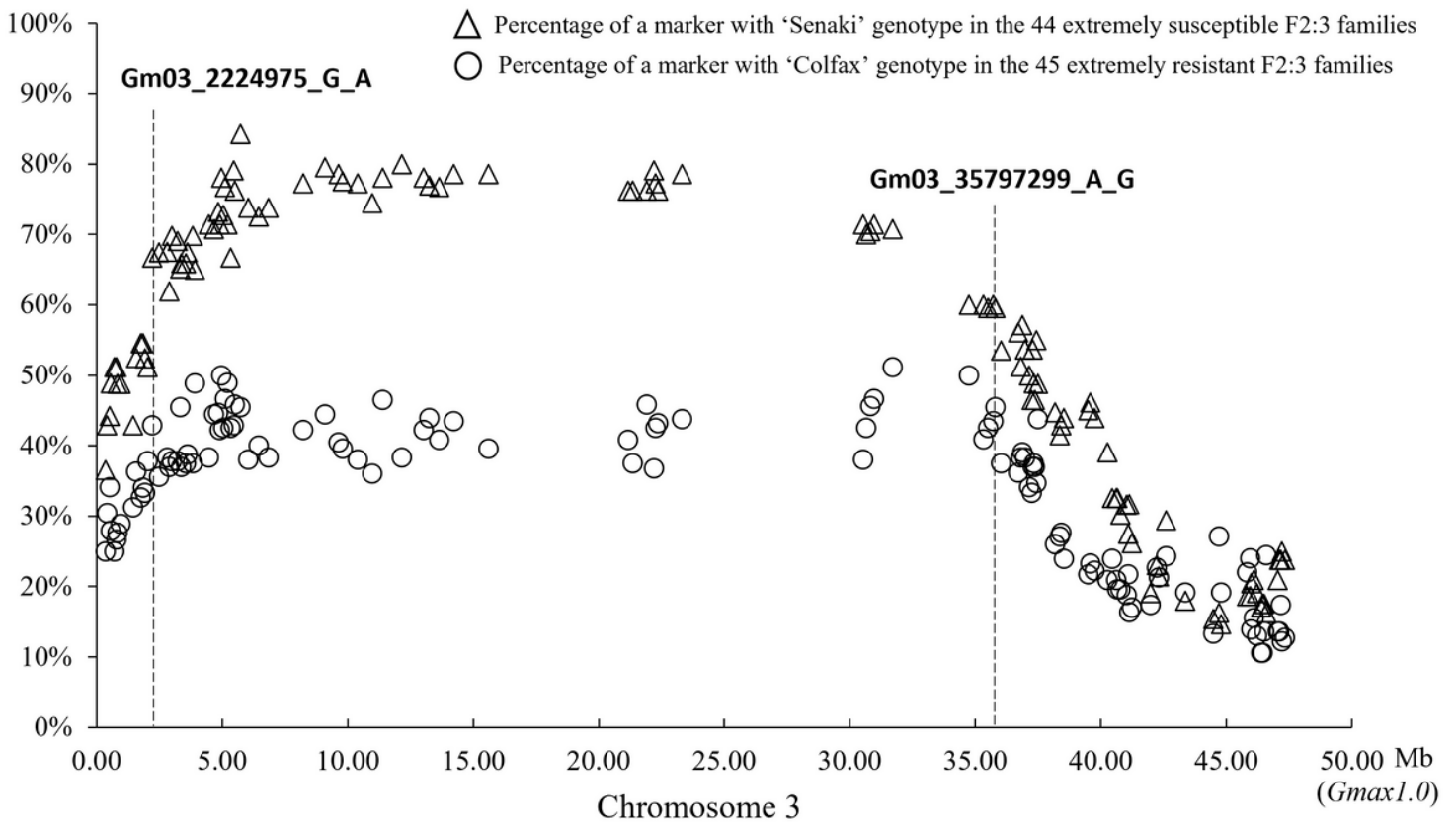


Figure 1

Linkage analysis of the resistance gene *Rpsan1* on chromosome 3 using the 89 extreme phenotypes of the F2:3 families derived from 'Colfax' × 'Senaki'. The dotted line indicates the position of left and right end of markers (Gm03_2224975_G_A and Gm03_35797299, respectively) with more than 60% of 'Senaki' genotype out of the 44 extremely susceptible F2:3 families.

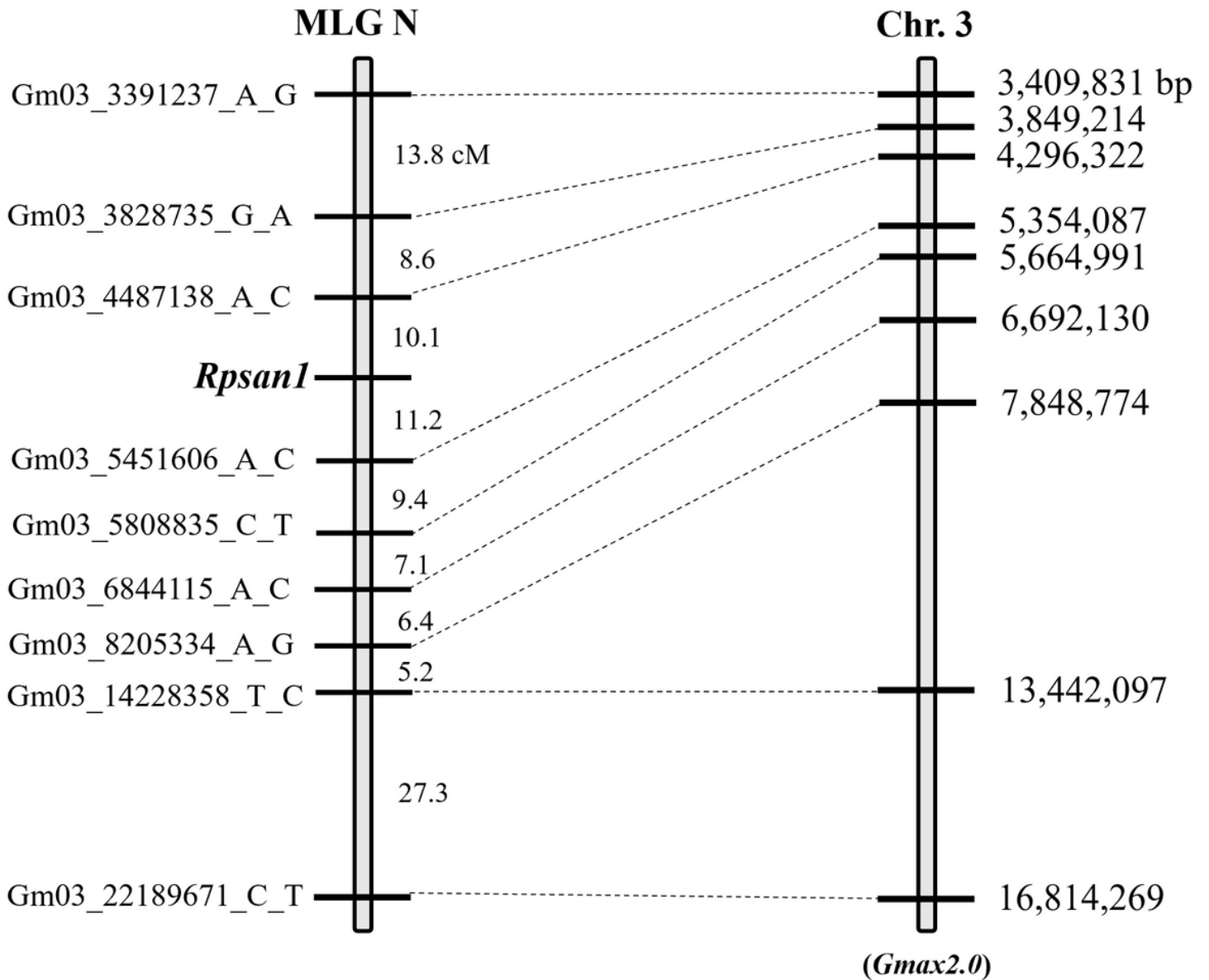


Figure 2

Genetic mapping of *Rpsan1* using 9 SNP markers on chromosome 3. Left: genetic position of the 8 SNP markers and the *Rpsan1* gene. Right: Physical position (*Gmax2.0*) of the mapped region of *Rpsan1*.

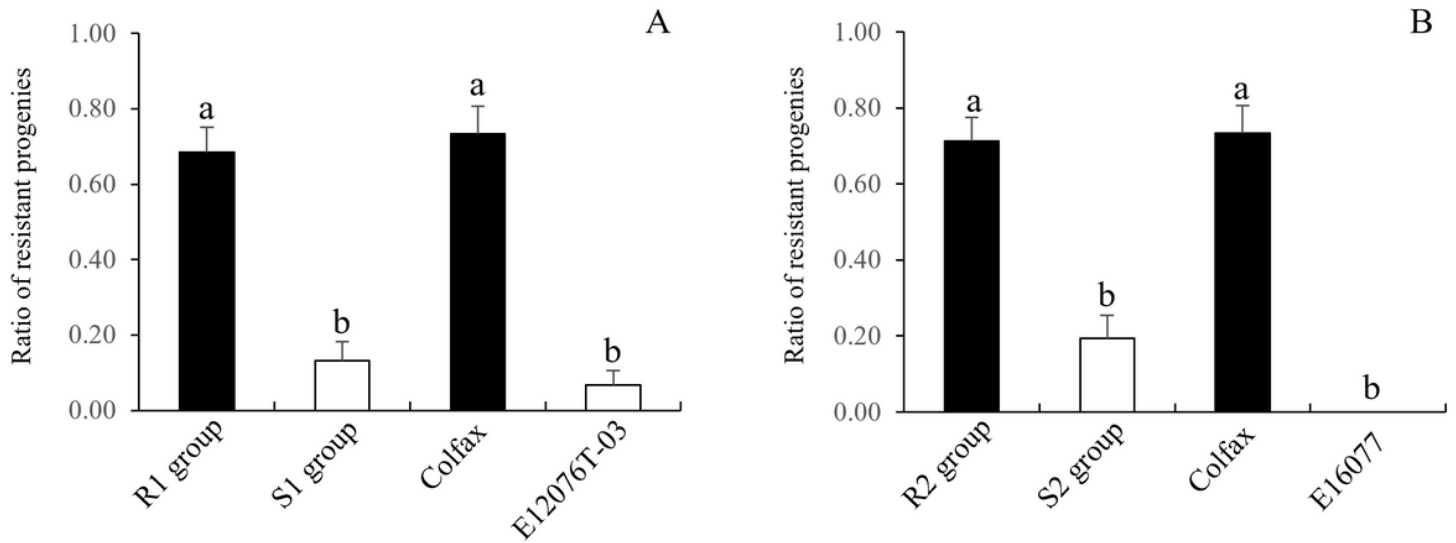


Figure 3

Validation of the *Rpsan1* locus using two breeding populations POP200032 (A), derived from E12076T-03 × Colfax, and POP200040 (B), derived from E16077 × Colfax. For POP200032, 19 F2:3 families were identified as '*Rpsan1/Rpsan1*' genotypes (R group) and 19 F2:3 families were identified as '*rpsan1/rpsan1*' genotypes (S group) using Gm03_5808835_C_T and Gm03_8205334_A_G. For POP200040, 16 F2:3 families were identified as '*Rpsan1/Rpsan1*' genotypes (R group) and 21 F2:3 families were identified as '*rpsan1/rpsan1*' genotypes (S group) using Gm03_4487138_A_C and Gm03_5808835_C_T. **A:** One-Way ANOVA test for the ratio of resistant progenies against *P. sansomeana* isolate MPS17-22 in POP200032, different letters indicate significant difference between groups at 0.05 level. **B:** One-Way ANOVA test for the ratio of resistant progenies against *P. sansomeana* isolate MPS17-22 in POP200040, different letters indicate significant difference between groups at 0.05 level.

Supplementary Files

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