

Loci and candidate gene identification for resistance to *Sclerotinia sclerotiorum* in soybean (*Glycine max* L. Merr.) via association and linkage maps

Xue Zhao^{1,†}, Yingpeng Han^{1,†}, Yinghui Li², Dongyuan Liu³, Mingming Sun^{1,4}, Yue Zhao¹, Chunmei Lv^{1,5}, Dongmei Li¹, Zhijiang Yang³, Long Huang³, Weili Teng¹, Lijuan Qiu^{2,*}, Hongkun Zheng^{3,*} and Wenbin Li^{1,*}

¹Key Laboratory of Soybean Biology in Chinese Ministry of Education (Key Laboratory of Soybean Biology and Breeding/Genetics of Chinese Agriculture Ministry), Northeast Agricultural University, 150030 Harbin, China,

²Institute of Crop Science, National Key Facility for Crop Gene Resources and Genetic Improvement (NFCRI), Chinese Academy of Agricultural Sciences, 100081 Beijing, China,

³Bioinformatics Division, Biomarker Technologies Corporation, 101300 Beijing, China,

⁴Information Center, Heilongjiang Academy of Agricultural Sciences, 150086 Harbin, China, and

⁵Institute of Plant Protection, Sichuan Academy of Agricultural Sciences, 610066 Chengdu, China

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*For correspondence (e-mails wenbinli@neau.edu.cn; qjulijuan@caas.cn; zhenghk@biomarker.com.cn).

†These authors have contributed equally to this work.

SUMMARY

Soybean white mold (SWM), caused by *Sclerotinia sclerotiorum* ((Lib.) W. Phillips), is currently considered to be the second most important cause of soybean yield loss due to disease. Research is needed to identify SWM-resistant germplasm and gain a better understanding of the genetic and molecular basis of SWM resistance in soybean. Stem pigmentation after treatment with oxaloacetic acid is an effective indicator of resistance to SWM. A total of 128 recombinant inbred lines (RILs) derived from a cross of 'Maple Arrow' (partial resistant to SWM) and 'Hefeng 25' (susceptible) and 330 diverse soybean cultivars were screened for the soluble pigment concentration of their stems, which were treated with oxalic acid. Four quantitative trait loci (QTLs) underlying soluble pigment concentration were detected by linkage mapping of the RILs. Three hundred and thirty soybean cultivars were sequenced using the whole-genome encompassing approach and 25 179 single-nucleotide polymorphisms (SNPs) were detected for the fine mapping of SWM resistance genes by genome-wide association studies. Three out of five SNP markers representing a linkage disequilibrium (LD) block and a single locus on chromosome 13 (Gm13) were significantly associated with the soluble pigment content of stems. Three more SNPs that represented three minor QTLs for the soluble pigment content of stems were identified on another three chromosomes by association mapping. A major locus with the largest effect on Gm13 was found both by linkage and association mapping. Four potential candidate genes involved in disease response or the anthocyanin biosynthesis pathway were identified at the locus near the significant SNPs (<60 kbp). The beneficial allele and candidate genes should be useful in soybean breeding for improving resistance to SWM.

Keywords: soybean white mold, *Sclerotinia sclerotiorum*, QTL mapping, GWA mapping, resistance genes, stem pigmentation.

INTRODUCTION

White mold, caused by *Sclerotinia sclerotiorum* ((Lib.) W. Phillips.) de Bary, is a devastating disease of many economically important crops (Boland and Hall, 1994). Soybean white mold (SWM) was first identified in Ontario, Canada in 1946 (Koch and Hidebrand, 1946; Hildebrand, 1948). Since 1978 the frequency of SWM outbreaks

has increased, spreading over the entire world (Cline and Jacobsen, 1983; Boland and Hall, 1986). Its widespread occurrence has been attributed to the rotation of soybean in fields with other crops susceptible to SWM (Grau *et al.*, 1982; Phipps and Porter, 1982), the favorable climates in the northern soybean growing regions (higher humidity,

cooler temperatures) and changes in cultivation practices such as decreased row spacing and/or the use of crop irrigation (Grau and Radke, 1984). Current strategies, such as increased row spacing and reduced irrigation before and during crop flowering and biocontrol, have not been effective in controlling this disease (Fernando *et al.*, 2005). The use of resistant cultivars is an effective and economic approach to coping with this disease. Hence investigation of the genetic control of SWM is fundamental for breeding soybean cultivars resistant to SWM.

The genetics of differential resistance among soybean cultivars suggests a multi-locus model (Kim and Diers, 2000). Currently, only partially resistant cultivars have been used for genetic mapping because of the lack of immune type resistance. A numbers of genetic loci and molecular markers have been found to be related to disease resistance (Collard and Mackill, 2008), but they were not used effectively in marker-assisted selection (MAS) to breed resistant varieties due to the low selection accuracy of large intervals and quantitative trait loci (QTLs) with a low genetic contribution (Collard and Mackill, 2008). Transformation of soybean with the germin gene, encoding the oligomeric protein oxalate oxidase (OxO), which oxidizes oxalic acid to carbon dioxide and hydrogen peroxide, does provide an effective means of controlling this disease, but the resistance to SWM is still low (Donaldson *et al.*, 2001). Therefore, MAS-based breeding for SWM resistance is a potential alternative.

It is widely accepted that the key factor in pathogenesis of *S. sclerotiorum* is the secretion of oxalic acid that acts as an unspecific toxin (Godoy *et al.*, 1990; Cessna *et al.*, 2000; Kim *et al.*, 2008). In addition, the fungus secretes numerous extracellular enzymes (Bateman and Beer, 1965; Lumsden, 1976; Marciano *et al.*, 1982). The method for measuring soluble pigment(s) content in the stem, based on the oxalic acid reaction, was developed by Wegulo and colleagues, and produces consistent results in both controlled environmental and field conditions (Wegulo *et al.*, 1998; Li *et al.*, 2009a). The soluble pigment(s) content in the stem has been found to represent a component of resistance to SWM, and the effectiveness of this method has proved to be useful in screening for SWM resistance in both soybean and common bean (Wegulo *et al.*, 1998; Kolkman and Kelly, 2000).

Traditionally, QTLs are identified using cross-populations such as recombinant inbred lines (RILs) in which homozygous alternative alleles are segregated (Huang and Han, 2014). These mapping populations have a high power to detect QTLs because each allele is present in 50% of the recombinant lines; they have a relatively simple genetic background but they are time-consuming to develop. A new approach to using natural populations for QTL analysis is genome-wide association (GWA) mapping. This type of mapping takes advantage of the large number of his-

toric recombination events that have occurred within a set of related accessions (Rafalski, 2010). Genome-wide association mapping couples events with linked DNA polymorphisms in order to associate phenotypic diversity with a relatively small region of the genome. However, unlike RIL populations, where each allele is at a frequency of 0.5, rare alleles in samples of natural population will occur at a lower frequency, making it difficult to detect their phenotypic effect. Genome-wide association mapping has been successfully used in *Arabidopsis thaliana* (Atwell *et al.*, 2010; Li *et al.*, 2010; Horton *et al.*, 2012), rice (Huang *et al.*, 2010; Zhao *et al.*, 2011), maize (Kump *et al.*, 2011; Yang *et al.*, 2014), sorghum (Morris *et al.*, 2013) and soybean (Hao *et al.*, 2012; Bastien *et al.*, 2014; Hwang *et al.*, 2014; Mamidi *et al.*, 2014; Zhang *et al.*, 2014) for the identification of QTLs and candidate genes for various ecological and agricultural traits. However, few of these studies, if any, have verified the candidate genes underlying SWM resistance. In this study, the phenotypic data on the soluble pigment content of stems (based on the OxO reaction) of 128 soybean RILs and 330 soybean accessions were used to identify the QTLs and candidate genes associated with SWM resistance.

RESULTS

Phenotypic characteristics of SWM resistance in RILs and natural populations

Soybean resistance to SWM was reflected by the OD₅₁₈ value after treatment with 40 mM oxalic acid for 48 h. The distribution of OD₅₁₈ values in the soybean accessions is shown in Figure 1(a) and that from RILs in Figure 1(b). The distribution of OD₅₁₈ for the RILs was skewed and ranges from 0 to 0.121 with a mean of 0.036. The OD₅₁₈ values of the soybean accessions showed a similar range of 0.001–0.124 with a mean of 0.023, and the distribution was also skewed towards the most susceptible than the resistant genotypes. The OD₅₁₈ values of the resistant cultivar 'Maple Arrow' and the moderately susceptible cultivar 'Williams 82' were 0.10 and 0.06, respectively, while that for the susceptible cultivar 'Hefeng 25' was 0.0001, one of the lowest among both the RILs and the accessions.

Simple sequence repeat-based genotyping and QTL mapping for SWM resistance

A previous study reported that QTLs underlying soluble pigment in soybean stem after treatment with oxalic acid were located on linkage group (LG) D1a (Gm01), A2 (Gm08), and B1 (Gm11) using the RIL population derived from the cross of 'Hefeng 25' and 'Maple Arrow' (Li *et al.*, 2009a). The number of known QTLs underlying soybean resistance to pathogenic fungus on LGs A2 (Gm08) and F (Gm13) was more than that of the other LGs according to the Consensus 4.0 genetic map of soybean in the SoyBase

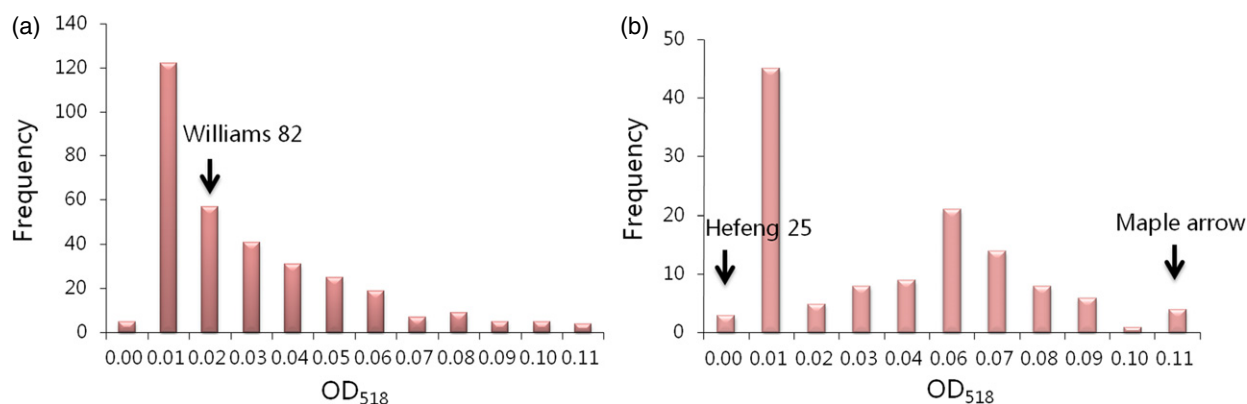


Figure 1. Histograms of soluble pigments in soybean stems.
(a) Distribution of soluble pigments in soybean stems among soybean germplasms.
(b) Distribution of soluble pigments in soybean stems among soybean recombinant inbred lines (RILs).

database (<http://www.soybase.org>). The results showed that 108 out of 431 simple sequence repeat (SSR) markers on the above four chromosomes were polymorphic between the two parents with a single PCR product, and 78 of them were clearly polymorphic in the progenies. All the 78 markers covering the four targeted soybean chromosomes (Gm01, Gm08, Gm11 and Gm13) were used to construct a genetic linkage map. Our genetic linkage map covered 462.99 cM of the soybean genome. The average distances between markers on Gm01, Gm08, Gm11 and Gm13 were 6.4, 5.5, 7.7 and 4.7 cM, respectively (Figure 2a). Four regions on Gm01, Gm08, Gm11 and Gm13 were significantly associated with the OD₅₁₈ of soluble pigment in soybean RILs. The four regions were between SSR01_0884 and SSR01_1102 (1.98 cM) on Gm01, between SSR08_1160 and SSR08_1127 (3.85 cM) on Gm08, between Sat_095 and SSR11_1208 (3.75 cM) on Gm11, and between SSR13_0114 and SSR13_0197 (0.92 cM) on Gm13. Each QTL explained 7.7–23.3% of the phenotypic variation (Table 1). Qswm13-1 on Gm13 near SSR13_0197 was a major QTL with the largest effect of genetic contribution and the minimum confidence interval. The beneficial allele of this QTL was from resistant parent 'Maple Arrow'.

Single-nucleotide polymorphism-based genotyping for 330 soybean accessions

For the further fine mapping of the SWM resistance genes and the investigation of the beneficial haplotypes in soybean germplasm, a haplotype map was constructed for the 330 soybean accessions. To genotype each soybean accession, specific locus amplified fragment sequencing (SLAF-seq) was done with an Illumina Genome Analyzer II (Illumina Inc., <http://www.illumina.com/>) (Sun *et al.*, 2013). Over 253 million reads (each 45 bp) were generated for the 330 genotypes, encompassing 11.39 Gbp of the soybean genomic DNA sequence. For each accession, about 57 418 high-quality tags (or SLAFs) were identified from 202

million paired-end reads after sequence alignment with the Williams 82 reference genome (Table S1 in Supporting Information). The SLAFs that were used for calling SNPs had an average depth of 6.02-fold per individual among 330 accessions. A total of 148 094 SNPs were initially called for this set of lines. Of these a large proportion (83%) were found to have a minor allele frequency (MAF) <5%, leaving 25 179 SNPs at MAF ≥ 0.05 to be used for the analyses. The mean genomic distance between SNP tags was about 37.7 kbp.

The dataset of 25 179 SNP markers covered all 20 chromosomes (Figure 2b). The largest number of SNPs was found on chromosome 18 (2223 SNPs) followed by chromosome 15 (1815 SNPs), and the smallest number of SNPs was observed on chromosomes 11 (817 SNPs) and 12 (706 SNPs). The number of SNPs on each chromosome was consistent with the physical length of the respective chromosome. The longest and shortest chromosomes were mapped as Gm18 and Gm12, respectively (Schmutz *et al.*, 2010).

Structure of soybean natural population and GWA mapping for SWM resistance

The genome-wide mapping of SNPs generated from a soybean panel of 330 accessions (including landraces and elite lines) permitted the elucidation of the genetic basis for resistance to SWM through GWA mapping. Before conducting GWA mapping, a principal component analysis (PCA) was performed using 25 179 SNPs of the 330 lines in the GWA mapping panel to estimate the influence of population structure on the mapping results. Principal component 1 (PC1) explained 7.94% of the variation in the genotypic data, while PC2 and PC3 explained 5.20 and 2.57% of the variation, respectively. When observing the first three axes of the PCA, the testing accessions can be divided into two or three groups (Figure 2c–e), although some intermediate lines make the grouping less clear, indicating a low layer of population structure within the

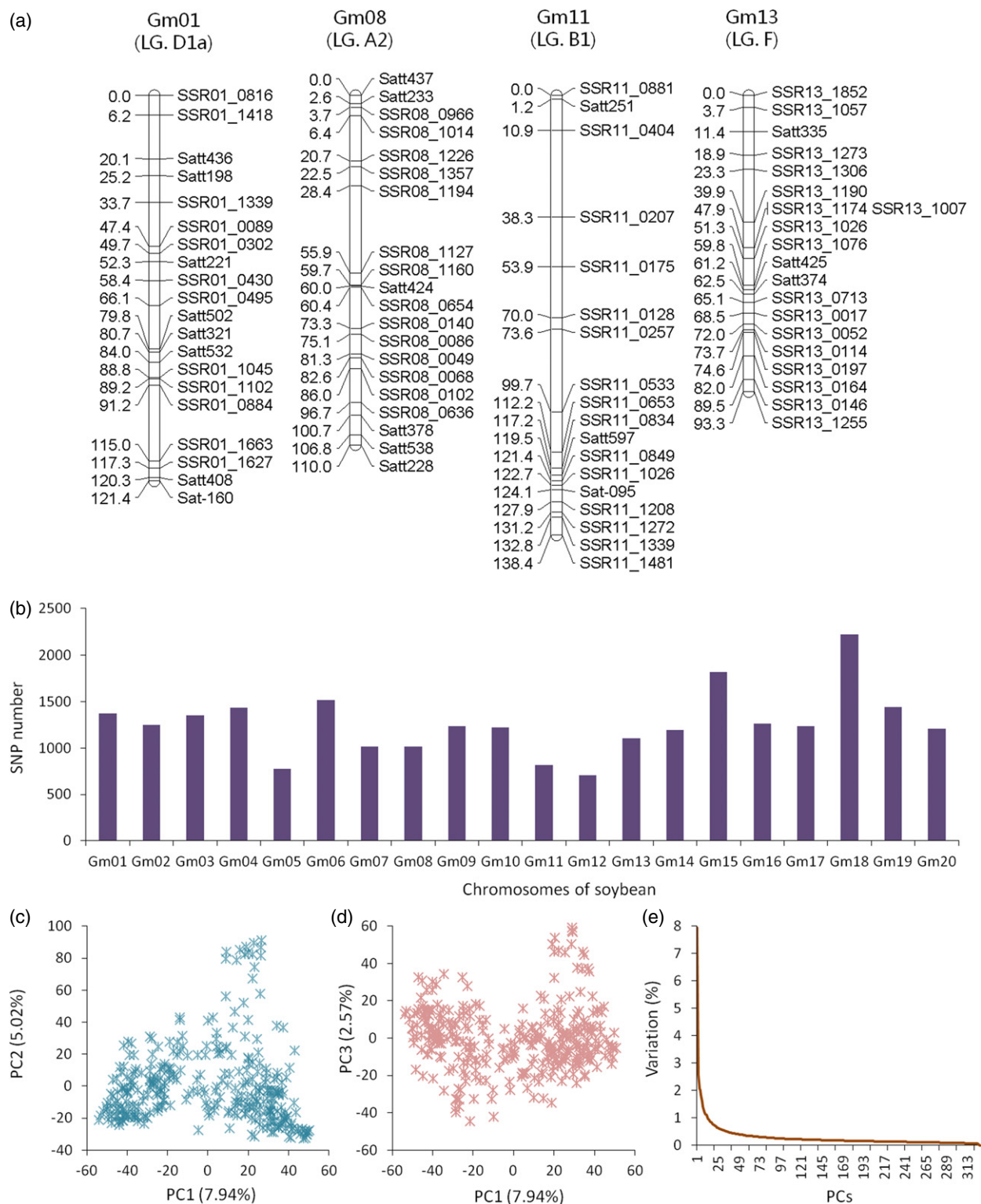


Figure 2. Genotyping and population structure of two mapping population.

(a) Simple sequence repeat-based genetic linkage map of soybean recombinant inbred line population.

(b) Single nucleotide polymorphism (SNP) distribution on 20 soybean chromosomes.

(c–e) Population structure of the soybean germplasm collection.

Table 1 Quantitative trait loci (QTLs) underlying stem soluble pigment contents reflecting the underlying part of the resistance of soybean to soybean white mold.

QTL	Molecular linkage group (Chromosome)	Marker interval	Confidence interval (cM)	LOD score	R^2 (%)
Qswm1-1	D1a (Gm01)	SSR01_0884~SSR01_1102	89.21–91.19	2.69	7.89
Qswm8-1	A2 (Gm08)	SSR11_1160~SSR11_1127	55.89–59.74	5.66	11.29
Qswm11-1	B1 (Gm11)	Sat-095~SSR11_1208	124.11–127.86	2.61	7.69
Qswm13-1	F (Gm13)	SSR13_0114~SSR13_0197	73.72–74.64	9.12	23.33

mapping panel. Hence, the first three PCs were added to the generalized linear model (GLM) and compressed mixed linear model (CMLM) for association analyses.

Our results showed that a total of 9, 38 and 38 SNPs were significantly associated with soluble pigment concentration with Naive, GLM and CMLM models, respectively. Among them, three were highly significantly associated with soluble pigment concentration by all three models (Figure 3a,b, Table 2). The association mapping results of GLM and Naive methods were almost the same. The CMLM method was more suitable for GWA mapping for SWM resistance, indicating that the effect of both population structure and kinship should be considered (Figure 3c). The first and second most significantly associated SNPs were common for all of the tested methods ($\alpha < 0.01$). The first peak SNP was found at Gm13: 3 296 576, which explained 23.62% of the total observed variation in soluble pigment concentration. The second peak SNP was found at Gm13: 4 244 040, which explained 18.63% of the total observed variation in soluble pigment concentration. There were another three minor loci that was associated with soluble pigment concentration through GLM and CMLM methods, found on Gm06, Gm10 and Gm11, respectively ($\alpha < 0.05$). No highly significant SNPs contributing to the soluble pigment concentration in soybean stems with large genetic effect was observed in any other region of the genome except for the association signal on Gm13. This result suggested that the causal gene linked with SNPs at Gm13 might be the major genetic locus responsible for natural variation in soluble pigment concentration in soybean stems after treatment with oxalic acid. Accessions with a cytosine (C) allele at the first peak SNP had, on average, a 67.6% higher OD_{518} value than the accessions with a thymine (T) allele. The minor allele (T) was represented in 49% of the 330 accessions. Accessions with the T allele at the second peak SNP had, on average, a 70.6% higher OD_{518} value than the accessions with a guanine (A) allele. The minor allele (T) was represented in 46% of the 330 accessions (Figure 3d).

A major locus on Gm13 and candidate genes potentially underlying disease resistance

The locus on Gm13 detected in both linkage analysis and GWA mapping could explain more than 20% of the varia-

tion of soluble pigment content in soybean stems, indicating that there might be a major gene in this genomic region (Figure 3e). To obtain a comprehensive view of the genomic landscape of the major locus on Gm13, linkage disequilibrium (LD) analysis of Gm13 was performed using 1103 SNPs. Linkage disequilibrium of Gm13 over physical distances is presented in Figure 3(f). Linkage disequilibrium blocks were distributed on Gm13 unevenly (from the point of view of the whole chromosome). The peak SNP associated with SWM resistance was located at one end of Gm13. Therefore, to understand the LD decay of the genomic regions Gm13: 0–8 mbp (4 mbp up- and downstream of the peak SNP) would help to determine candidate genes and beneficial haplotypes. The LD decay regression curve that was fitted to the LD plot fell below $R^2 = 0.2$ at about 0.133 mbp (Figure 3g). The first and second peak SNPs were located in a similar position on Gm13 but were not in the same LD block (Figure 3h). The first peak SNP (Gm13: 3 296 576) was involved in a 329-kbp long LD block that was encompassed by five SNP markers. The second peak SNP (Gm13: 4 244 040) was in a genomic region that was in a state of linkage equilibrium.

SSR13_1164, the left flanking marker of Qswm13-1 (the major QTL on Gm13 identified by linkage analysis based on soybean RILs), was located in the same LD block as the first peak SNP (Gm13: 3 296 576), while the right flanking marker of Qswm13-1, SSR13_1197, was between the first and second peak SNP and was nearer to the second peak SNP (Gm13: 4 244 040) at a distance of 244 kbp (Figure 3h). Overall, the genomic regions determined by association signals from GWA studies (GWAS) and QTL from linkage analysis overlapped. The interval of Qswm13-1 was divided by two peak SNPs due to the high mapping resolution of GWAS since the marker density of the soybean haplotype map was higher than that of genetic linkage map in this study. This result indicated that the first and second peak SNPs from GWAS might represent two different genomic regions controlling soybean resistance to SWM.

Within 300 kbp either side of the SNP Gm13: 3 296 576 and 4 244 040 (LD decay distance in the region) there were a total of 13 and 10 genes, respectively (Table 3). Eight of the genes around the peak SNP on linked genomic regions were without any definite annotation concerning their

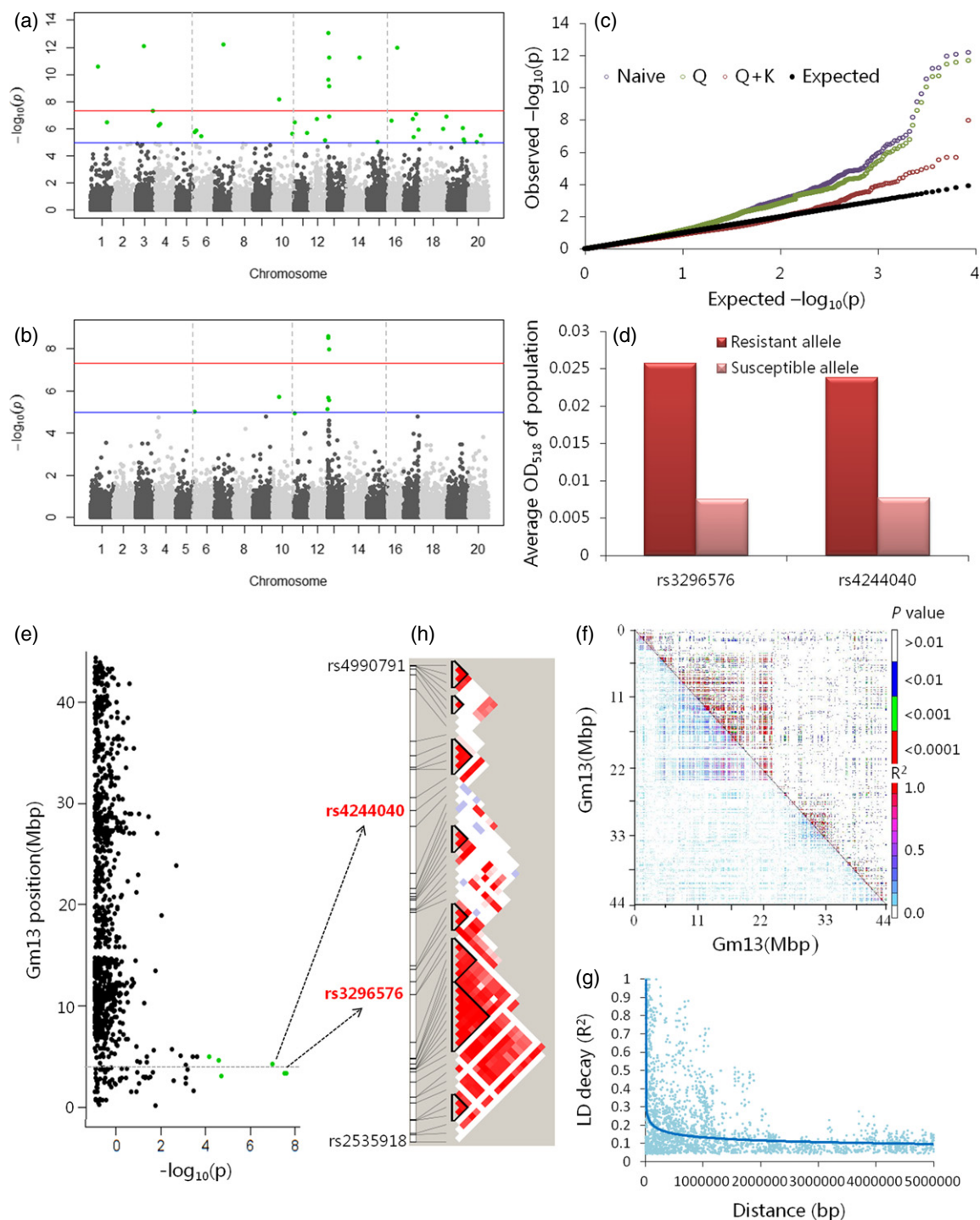


Figure 3. Genome-wide association scan for soluble pigments in soybean stems and genomic landscape of the major locus on Gm13.

(a, b) Genome-wide P -values from the generalized linear model (GLM) and compressed mixed linear model (CMLM). The x-axis shows the single nucleotide polymorphism (SNPs) along each chromosome; the y-axis is the $-\log_{10}(P)$ -value for the association. Colored dots in (a) and (b) indicate SNPs with P -values $< 1.99 \times 10^{-6}$ ($\alpha < 0.05$) in both models.

(c) Integration of the quantile–quantile plots for all the naive, generalized and mixed linear models for soluble pigment in all samples, respectively.

(d) Average OD_{518} value of accessions carrying the same allele of the significant SNPs on Gm13 in the natural soybean population.

(e) Association signals of OD_{518} values on Gm13.

(f) Linkage disequilibrium analysis of Gm13.

(g) The linkage disequilibrium (LD) decay of the 1–8 mbp genomic region of Gm13.

(h) The distribution of LD blocks of the major locus on Gm13 and the relationship between Qswm13-1 and peak SNPs.

Table 2 Single nucleotide polymorphisms (SNPs) associated with the plant absorbance value reflected by OD₅₁₈.

SNP	Position	$-\log_{10}(P)$		R^2		MAF	Beneficial allele
		MLM	GLM	MLM	GLM		
rs2707217	Gm06:2707217	5.03	5.78	7.39	8.06	0.06	G
rs15929904	Gm10:15929904	5.71	8.19	8.37	11.11	0.07	C
rs1808219	Gm11:1808219	4.94	6.50	7.28	9.03	0.07	T
rs3296576	Gm13:3296576	8.59	13.06	23.62	9.52	0.50	C
rs3296245	Gm13:3296245	8.51	13.06	22.56	9.52	0.49	A
rs4244040	Gm13:4244040	7.98	11.27	18.76	8.50	0.46	T
rs3446126	Gm13:3446126	5.67	9.63	17.20	6.96	0.31	G

GLM, generalized linear model; MLM, mixed linear model; MAF, minor allele frequency.
 P is the statistical P -value for the significance of the odds ratio in GWAS.

Table 3 Candidate genes within the linkage disequilibrium block or linked genomic region of single nucleotide polymorphisms (SNPs) most highly associated with soluble pigment in soybean stem.

Gene	Start	Stop	Direction	Distance to SNP (bp)	Anotation
Glyma13 g03332	3 266 692	3 269 757	Reverse	29 884	GDLS-like Lipase/Acylhydrolase superfamily protein
Glyma13 g03340	3 287 015	3 287 293	Forward	9561	
Glyma13 g03351	3 288 217	3 291 323	Forward	8359	
Glyma13 g03360	3 300 884	3 301 801	Reverse	4308	PR5-like receptor kinase
Glyma13 g03370	3 310 067	3 313 093	Reverse	13 491	
Glyma13 g03385	3 315 400	3 326 116	Reverse	18 824	C-8,7 sterol isomerase
Glyma13 g03400	3 358 533	3 366 701	Forward	61 957	3'-5' exonuclease domain-containing protein
Glyma13 g03405	3 396 752	3 397 400	Reverse	100 176	PQ-loop repeat family protein/transmembrane family protein
Glyma13 g03410	3 398 041	3 399 703	Reverse	101 465	ATP synthase alpha/beta family protein
Glyma13 g03421	3 408 738	3 414 656	Forward	112 162	Plant protein of unknown function (DUF869)
Glyma13 g03430	3 435 222	3 441 347	Forward	138 646	Pheophorbide a oxygenase family protein with Rieske [2Fe-2S] domain
Glyma13 g03920	4 139 185	4 147 410	Forward	104 855	Vacuolar protein sorting 26A
Glyma13 g03930	4 148 428	4 153 406	Reverse	95 612	mce-related protein
Glyma13 g03940	4 175 587	4 178 013	Forward	68 453	Peroxisomal membrane 22 kDa (Mpv17/PMP22) family protein
Glyma13 g03953	4 182 405	4 183 298	Reverse	68 453	
Glyma13 g03966	4 199 747	4 202 172	Reverse	61 635	Transducin/WD40 repeat-like superfamily protein
Glyma13 g03980	4 204 419	4 205 547	Reverse	39 621	
Glyma13 g03990	4 208 059	4 212 345	Reverse	35 981	Protein kinase 2A
Glyma13 g04000	4 224 687	4 225 600	Forward	19 353	
Glyma13 g04005	4 227 937	4 229 596	Reverse	16 103	RINT-1/TIP-1 family
Glyma13 g04010	4 228 086	4 229 583	Reverse	15 954	
Glyma13 g04020	4 245 201	4 250 227	Reverse	1161	myb domain protein 33
Glyma13 g04031	4 304 337	4 316 595	Forward	60 297	
Glyma13 g04041	4 344 811	4 346 283	Reverse	100 771	C ₂ H ₂ -type zinc finger family protein

biological functions. The domain of *Glyma13g03421* belongs to the domain of unknown function (DUF869). Of the rest, 14 genes that have domains of known function, four genes might be involved in the disease resistance response of plants. *Glyma13 g03360* was located 4.3 kbp from the first peak SNP with a kinase domain encoding a PR5-like receptor that was related to a family of protein-serine/threonine kinases involved in the expression of self-incompatibility and disease resistance (Wang *et al.*, 1996). The gene *Glyma13 g04020*, nearest to the second peak SNP (rs4244040), was located at a position of 1.6 kbp to the rs4244040 locus and might be a mem-

ber of the *RINT-1_TIP-1* family encoding a *Rad50*-interacting protein that participates in radiation-induced checkpoint control, as well as the *TIP-1* protein from yeast that seems to be involved in a complex with sec20p that is required for Golgi transport (Arasaki *et al.*, 2013). It is noteworthy that a *MYB* transcription factor gene was 60.3 kbp from the rs4244040 locus. Plant MYBs have been implicated in controlling pathways as diverse as secondary metabolism (including the anthocyanin pathway), development, signal transduction and disease resistance (Allan *et al.*, 2008; Lev-Yadun and Gould, 2009; Pierantoni *et al.*, 2010; Salvatierra *et al.*, 2010).

Anthocyanins belong to the diverse group of ubiquitous secondary metabolites known as flavonoids (Dubos *et al.*, 2008). In plants, flavonoids are believed to have a variety of functions, including defense and protection against light stress. The pigmented anthocyanin compounds play an important reproductive role as attractants in plant and animal interactions (Allan *et al.*, 2008).

DISCUSSION

Genetic mapping in crops is usually undertaken in segregating populations, such as F_2 populations, RILs or backcross inbred lines (Huang and Han, 2014). With the rapid development of sequencing technologies and computational methods, GWAS that take full advantage of ancient recombination events to identify the genetic loci underlying traits are becoming a powerful tool for detecting natural variation underlying complex traits in crops (Rafalski, 2010). The results from the two kinds of mapping methods could provide mutual authentication.

The population sizes in previous GWAS on soybean were one to two hundred, including soybean landraces and/or elite lines (Hao *et al.*, 2012; Bastien *et al.*, 2014; Hwang *et al.*, 2014; Mamidi *et al.*, 2014). Genotyping approaches included genotyping by sequencing (GBS), Golden Gate and gene microarray (Hao *et al.*, 2012; Bastien *et al.*, 2014; Hwang *et al.*, 2014; Mamidi *et al.*, 2014). The marker density and uniform distribution should be considered during high-resolution GWAS, no matter which genotyping method is used. The appropriate marker density for GWAS depends on the linkage disequilibrium of the genomes. Previous studies showed that the average distance of LD decay in the cultivated soybean genome was about 150 kbp (Lam *et al.*, 2010; Li *et al.*, 2013). Hwang *et al.* (2014) used 30 000 SNPs that were identified from 298 soybean accessions to evaluate the LD level and found that the mean LD (R^2) rapidly declined to 0.2 within 360 kbp in euchromatic regions and declined to 0.2 at 9600 kbp in heterochromatic regions. In the present study, a SNP marker density of one SNP per 37.7 kbp was obtained, which can meet the demand of GWA mapping of multiple soybean traits.

The soybean resistance to SWM is complex in its pathogenic mechanism and resistance pathway and is a quantitative trait (Kim and Diers, 2000; Vuong *et al.*, 2008). The distribution of stem rot length via the method of direct inoculation by *S. sclerotiorum* follows a normal distribution in both the natural soybean population and in RILs (Arahana *et al.*, 2001; Bastien *et al.*, 2014), indicating that the length of stem rot by direct inoculation reflects the comprehensive resistance of tested soybean germplasm or RILs to SWM, corresponding to the complexity of genetic control for SWM resistance. To understand the genetic mechanism of resistance to the disease, the expression of soybean response to SWM

can be disassembled to transform a quantitative character into several qualitative traits or qualitative-like traits according to the known pathway of SWM resistance. Oxalic acid, a toxic secretion from *S. sclerotiorum*, is widely accepted as a key factor in disease pathogenesis (Godoy *et al.*, 1990; Cessna *et al.*, 2000; Kim *et al.*, 2008). In the present study, the toxicity of oxalic acid to soybean was reflected by the amount of soluble pigment in stems (OD_{518}). The distribution of OD_{518} in RILs and the natural soybean population was skewed, with few highly resistant types. Most of individuals were susceptible, indicating that part of soybean resistance to SWM determined by pathogenic oxalate might be an approximately qualitative trait.

In total, four QTLs from linkage analysis and five association signals from GWA mapping underlying SWM resistance, distributed on six soybean chromosomes, were detected. The major locus on Gm13 was identified by both linkage and GWA mapping, but was not found in a GWAS reported by Bastien *et al.* (2014) with 150 individuals (Bastien *et al.*, 2014). The marker interval of *Qswm8-1* overlapped with the QTL that were reported by Guo *et al.* (2008) and Arahana *et al.* (2001) based on the phenotype of soybean stem rot (Figure 4) (Arahana *et al.*, 2001; Guo *et al.*, 2008). This result suggests that choosing multiple methods to measure soybean resistance to SWM according to different pathogenic pathways could help us find more major loci underlying resistance to SWM.

In addition, in the present study a relationship of linkage or overlap was not found among the QTLs and association signals except for the major locus on Gm13. All the SWM resistance-related QTLs from linkage analysis were from the resistant parent 'Maple Arrow'; however, the different resistance signals from GWA mapping were from diverse resistant donors. Therefore, new haplotypes associated with SWM resistance can be obtained from GWA mapping rather than from linkage analysis.

CONCLUSIONS

A high-density haplotype map of SNP markers with unprecedented sample size were obtained through SLAF-seq technology. The genotype dataset allowed us to investigate the genetic control of multiple important soybean traits via a GWA mapping approach. In total, eight regions associated with SWM resistance by QTL mapping and GWA mapping were identified. Among them, a consistent locus on Gm13 was detected by the two mapping approaches. Three candidate genes in the LD block or linkage region of significant SNP markers might be involved in resistance of soybean to SWM. The beneficial allele and candidate genes should be useful in soybean breeding for improving resistance to SWM through the molecular design approach.

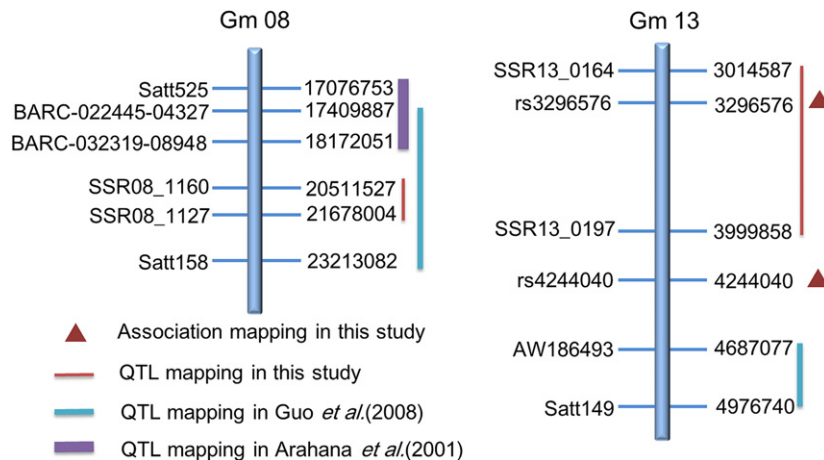


Figure 4. Overlapping or linkage relationships among quantitative trait loci (QTLs) in previous and present studies.

EXPERIMENTAL PROCEDURES

Mapping population

Two mapping populations were used in the present study. One was a natural population consisting of 330 diverse soybean accessions to represent the genetic diversity inside and outside China. The accessions were selected from the 2000 core germplasms in China. For Chinese accessions, diverse soybean landraces (105) and elite cultivars (201) were included. The germplasms covered the regions of China from 19 to 48° N and from 74 to 128° E (Table S2). Twenty-four soybean cultivars from outside China were also included. Among the 330 germplasms, soybean cultivars with good and poor resistance to *S. sclerotiorum* were involved, including 'Maple Arrow' (a partially resistant cultivar), 'Williams 82' (a moderately susceptible cultivar) and 'Hefeng 25' (a susceptible cultivar) (Li *et al.*, 2009a; Bastien *et al.*, 2014). The RIL population, consisting of 128 F_{5:10} RILs, was derived from the cross of 'Hefeng 25' and 'Maple Arrow'.

Phenotypic evaluation of SWM resistance

The soybean accessions and RILs were grown in the greenhouse in 13-cm diameter pots (containing a ready-made peat-based growing mix with 75–85% soil) with five plants representing a feeding block. For each RIL or accession the feeding block had three blocks per replication. A randomized block design with three replications was used. The soluble pigment content of soybean stem was measured using the method described by Wegulo *et al.* (1998). At the V1 or V2 growth stage, the plant stem was severed approximately 0.5 cm above the level of the soil, completely defoliated and placed immediately into 13 mm × 9 mm × 100 mm tubes containing 5 ml of 40 mM oxalic acid (one stem per tube). The plant absorbance value was measured for five plants in each bucket within each RIL and soybean cultivar replicate. Tubes containing soybean stems were arranged vertically in test-tube racks and incubated at 20°C under a 12-h/12-h (light/dark) photoregime. After 48 h, 4 ml of the OxO in each test tube was transferred to a 5-ml cup, and the plant absorbance value at OD₅₁₈ was determined in a Perkin Lambda 35 spectrophotometer at 518 nm (Perkin-Elmer, <http://www.perkinelmer.com/>). The data for plant absorbance value reflected by OD₅₁₈ in the two mapping populations were used as phenotypic data for QTL and association mapping.

Specific locus amplified fragment sequencing and SNP calling

For each soybean accession, genomic DNA was isolated from fresh leaves of a single plant per accession at the V3 stage using the methods described by Wu *et al.* (2010). Genomic DNA was analyzed according to SLAF-seq (Sun *et al.*, 2013). Sequencing libraries of each accession were constructed through double digestion with the restriction enzymes *MseI* and *HaeIII* (Thermo Fisher Scientific Inc., <http://www.thermofisher.com/>) that can digest soybean genomic DNA into more than 50 000 sequencing tags of length 300–500 bp. The tags were evenly distributed in unique regions of the genome. The 45-bp sequence read at both ends of the fragment in each library was generated by Illumina Genome Analyzer II (Illumina Inc.), using a barcode approach to identify each sample.

The SHORT OLIGONUCLEOTIDE ALIGNMENT PROGRAM 2 (SOAP2) was used to map raw paired-end reads onto the reference genome (Glycine_max_Williams_82 8x release v1.01) (Li *et al.*, 2009b). The SLAF groups were generated by reads that were mapped to the same position. If an accession was only partly digested by the restriction enzymes, some reads mapped to the reference genome should have overlaps with two SLAF tags. Such reads were assigned to both SLAF tags in that accession. A total of 60 000 high-quality SLAF tags were obtained from each of the 330 genotypes.

For SNP calling, the MAF threshold was set at 0.05. If the depth of the minor allele was larger than a third of the total sample depth, the genotype was regarded as heterozygous.

Simple sequence repeat-based genetic linkage map and QTL analyses

Young trifoliate leaf tissues were collected from the 128 grown F_{5:8} individuals of the RIL population and the parents that were planted in the same conditions as above. Soybean genomic DNA was isolated as described previously by Wu *et al.* (2010). The genomic DNA from each of the RILs and parents was genotyped using publicly available markers (Cregan *et al.*, 1999; Song *et al.*, 2004, 2010). Amplification using microsatellite polymerase chain reaction (PCR) primer sets was conducted as described by Li *et al.* (2009a) and Cregan *et al.* (1999). In brief, the PCR mixture con-

tained 20 ng of total genomic DNA, 1 × PCR buffer [10 mM 2-amino-2-(hydroxymethyl)-1,3-propanediol (TRIS)-HCl, 50 mM KCl, 2.5 mM Mg²⁺, pH 8.3], 100 nM of each forward/reverse primer, 0.16 mM of each dNTP and 0.25 units of Taq polymerase (Sangon Biotechnology Company, <http://www.life-biotech.com/>) for a total volume of 20 µl. Standard PCR was conducted as follows: a denaturation step at 94°C for 5 min, 35 cycles at 94°C for 30 sec, 47–58°C for 30 sec and 72°C for 30 sec and an extension step at 72°C for 5 min, and followed by a 10°C soak. The PCR products were separated by either 6% (w/v) denatured polyacrylamide gel electrophoresis (PAGE) with silver staining or 2–3% (w/v) agarose gel electrophoresis with ethidium bromide staining.

Genetic map distances were calculated using the MAPMAKER 3.0b program (Lander *et al.*, 2009), at a log likelihood of 5.0, with a maximum Haldane distance of 50 cM. The QTL were calculated using the CIM-ADD method of WINQTL/CARTOGRAPHER 2.5 with a log likelihood of 2.5 as the threshold of existing QTLs (Wang *et al.*, 2012).

Population structure and linkage disequilibrium

The structure of the natural soybean population was analyzed using a PCA approach with the GAPIT software package (Lipka *et al.*, 2012). The whole and partial LD of Gm13 was calculated between each pair of SNPs (R^2) using HAPLOVIEW 4.2 (Barrett *et al.*, 2005). Parameters in the program included MAF (≥ 0.05) and the integrity of each SNP ($\geq 50\%$).

Association mapping

A total of 25 179 SNPs from 330 soybean accessions were used for association analysis with Naive Model, GLM and CMLM in GAPIT (Lipka *et al.*, 2012). The P -value that was adjusted with the Bonferroni method at the $\alpha \leq 0.01$ and 0.05 level (corresponding $P \leq 3.97 \times 10^{-7}$ and 1.99×10^{-6} , respectively) was used as the threshold to determine whether a significant association existed (Holm, 1979). Candidate genes that were located within the LD block in which a peak SNP was involved were identified.

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AUTHORS' CONTRIBUTIONS

WBL designed and supervised the research; XZ and YPH analysed the data, XZ, MMS, YZ and CML conducted the field trial to evaluate SWM resistance. DYI and HKZ, ZJY and LH performed the genome sequencing; WLT, YHL and DML constructed genetic map; XZ, YPH, LJQ and WBL

wrote the manuscript. All authors read and approved the manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Table S1. Sequencing and single nucleotide polymorphism naming criteria used.

Table S2. Information on 330 soybean germplasms.

REFERENCES

- Allan, A.C., Hellens, R.P. and Laing, W.A. (2008) MYB transcription factors that colour our fruit. *Trends Plant Sci.* **13**, 99–102.
- Arahana, V.S., Graef, G.L., Specht, J.E., Steadman, J.R. and Eskridge, K.M. (2001) Identification of QTLs for resistance to *Sclerotinia sclerotiorum* in soybean. *Crop Sci.* **41**, 180–188.
- Arasaki, K., Takagi, D., Furuno, A., Sohda, M., Misumi, Y., Wakana, Y., Inoue, H. and Tagaya, M. (2013) A new role for RINT-1 in SNARE complex assembly at the trans-Golgi network in coordination with the COG complex. *Mol. Biol. Cell* **24**, 2907–2917.
- Atwell, S., Huang, Y.S., Vilhjalmsen, B.J. *et al.* (2010) Genome-wide association study of 107 phenotypes in *Arabidopsis thaliana* inbred lines. *Nature*, **465**, 627–631.
- Barrett, J.C., Fry, B., Maller, J. and Daly, M.J. (2005) Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics*, **21**, 263–265.
- Bastien, M., Sonah, H. and Belzile, F. (2014) Genome wide association mapping of resistance in soybean with a genotyping-by-sequencing approach. *The Plant Genome*, **7**, 1–13.
- Bateman, D.F. and Beer, S.V. (1965) Simultaneous production and synergistic action of oxalic acid and polygalacturonase during pathogenesis by *Sclerotium rolfsii*. *Phytopathology*, **55**, 204–211.
- Boland, G.J. and Hall, R. (1986) Growth room evaluation of soybean cultivars for resistance to *Sclerotinia sclerotiorum*. *Can. J. Plant Sci.* **66**, 559–564.
- Boland, G.J. and Hall, R. (1994) Index of plant hosts of *Sclerotinia sclerotiorum*. *Can. J. Plant Pathol.* **16**, 93–108.
- Cessna, S.G., Sears, V.E., Dickman, M.B. and Low, P.S. (2000) Oxalic acid, a pathogenicity factor for *Sclerotinia sclerotiorum*, suppresses the oxidative burst of the host plant. *The Plant Cell Online*, **12**, 2191–2199.
- Cline, M.N. and Jacobsen, B.J. (1983) Methods for evaluating soybean cultivars for resistance to *Sclerotinia sclerotiorum*. *Plant Dis.* **67**, 784–786.
- Collard, B.C.Y. and Mackill, D.J. (2008) Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **363**, 557–572.
- Cregan, P.B., Jarvik, T., Bush, A.L. *et al.* (1999) An integrated genetic linkage map of the soybean genome. *Crop Sci.* **39**, 1464–1490.
- Donaldson, P.A., Anderson, T., Lane, B.G., Davidson, A.L. and Simmonds, D.H. (2001) Soybean plants expressing an active oligomeric oxalate oxidase from the wheat gf-2.8 (germin) gene are resistant to the oxalate-secreting pathogen *Sclerotinia sclerotiorum*. *Physiol. Mol. Plant Pathol.* **59**, 297–307.
- Dubos, C., Le Gourrierec, J., Baudry, A., Huep, G., Lanet, E., Debeaujon, I., Routaboul, J.-M., Alboresi, A., Weisshaar, B. and Lepiniec, L. (2008) MYBL2 is a new regulator of flavonoid biosynthesis in *Arabidopsis thaliana*. *Plant J.* **55**, 940–953.
- Fernando, W.G.D., Ramarathnam, R., Krishnamoorthy, A.S. and Savchuk, S.C. (2005) Identification and use of potential bacterial organic antifungal volatiles in biocontrol. *Soil Biol. Biochem.* **37**, 955–964.
- Godoy, G., Steadman, J.R., Dickman, M.B. and Dam, R. (1990) Use of mutants to demonstrate the role of oxalic acid in pathogenicity of *Sclerotinia sclerotiorum* on *Phaseolus vulgaris*. *Physiol. Mol. Plant Pathol.* **37**, 179–191.

- Grau, C.R. and Radke, V.L. (1984) Effects of cultivars and cultural practices on Sclerotinia stem rot of soybean. *Plant Dis.* **68**, 56–58.
- Grau, C.R., Radke, V.L. and Gillespie, F.L. (1982) Resistance of soybean cultivars to *Sclerotinia sclerotiorum*. *Plant Dis.* **66**, 506–508.
- Guo, X.M., Wang, D.C., Gordon, S.G., Helliwell, E., Smith, T., Berry, S.A., Martin, S.K.S. and Dorrance, A.E. (2008) Genetic mapping of QTLs underlying partial resistance to *Sclerotinia sclerotiorum* in soybean PI 391589A and PI391589B. *Crop Sci.* **48**, 1129–1139.
- Hao, D., Chao, M., Yin, Z. and Yu, D. (2012) Genome-wide association analysis detecting significant single nucleotide polymorphisms for chlorophyll and chlorophyll fluorescence parameters in soybean (*Glycine max*) landraces. *Euphytica*, **186**, 919–931.
- Hildebrand, A.A. (1948) Soybean diseases in Ontario. *Soybean Digest*. **10**, 16–17.
- Holm, S. (1979) A simple sequentially rejective multiple test procedure. *Scand. J. Stat.* **6**(2), 65–70.
- Horton, M.W., Hancock, A.M., Huang, Y.S. et al. (2012) Genome-wide patterns of genetic variation in worldwide *Arabidopsis thaliana* accessions from the RegMap panel. *Nat. Genet.* **44**, 212–216.
- Huang, X.H. and Han, B. (2014) Natural variations and genome-wide association studies in crop plants. *Annu. Rev. Plant Biol.* **65**, 531–551.
- Huang, X., Wei, X., Sang, T. et al. (2010) Genome-wide association studies of 14 agronomic traits in rice landraces. *Nat. Genet.* **42**, 961–967.
- Hwang, E.Y., Song, Q., Jia, G., Specht, J.E., Hyten, D.L., Costa, J. and Cregan, P.B. (2014) A genome-wide association study of seed protein and oil content in soybean. *BMC Genom.* **15**, 1.
- Kim, H.S. and Diers, B.W. (2000) Inheritance of partial resistance to sclerotinia stem rot in soybean research supported by the michigan agricultural experiment station and grants from the michigan soybean promotion committee. *Crop Sci.* **40**, 55–61.
- Kim, K.S., Min, J.-Y. and Dickman, M.B. (2008) Oxalic acid is an elicitor of plant programmed cell death during *Sclerotinia sclerotiorum* disease development. *Mol. Plant Microbe Interact.* **21**, 605–612.
- Koch, L.M. and Hidebrand, A.A. (1946) Soybean diseases in south-western Ontario in 1946. *Can Plant Dis. Sur.* **26**, 27–28.
- Kolkman, J.M. and Kelly, J.D. (2000) An indirect test using oxalate to determine physiological resistance to white mold in common bean. *Crop Sci.* **40**, 281–285.
- Kump, K.L., Bradbury, P.J., Wissner, R.J. et al. (2011) Genome-wide association study of quantitative resistance to southern leaf blight in the maize nested association mapping population. *Nat. Genet.* **43**, 163–168.
- Lam, H.M., Xu, X., Liu, X. et al. (2010) Resequencing of 31 wild and cultivated soybean genomes identifies patterns of genetic diversity and selection. *Nat. Genet.* **42**, 1053–1059.
- Lander, E.S., Green, P., Abrahamson, J., Barlow, A., Daly, M.J., Lincoln, S.E. and Newberg, L.A. (2009) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations (vol 1 pg 174, 1987). *Genomics*, **93**, 398–398.
- Lev-Yadun, S. and Gould, K. (2009) Role of anthocyanins in plant defence. In *Anthocyanins* (Winefield, C., Davies, K. and Gould, K., eds). New York: Springer New York, pp. 22–28.
- Li, D., Sun, M., Han, Y., Teng, W. and Li, W. (2009a) Identification of QTL underlying soluble pigment content in soybean stems related to resistance to soybean white mold (*Sclerotinia sclerotiorum*). *Euphytica*, **172**, 49–57.
- Li, R., Yu, C., Li, Y., Lam, T.-W., Yiu, S.-M., Kristiansen, K. and Wang, J. (2009b) SOAP2: an improved ultrafast tool for short read alignment. *Bioinformatics*, **25**(15), 1966–1967.
- Li, Y., Huang, Y., Bergelson, J., Nordborg, M. and Borevitz, J.O. (2010) Association mapping of local climate-sensitive quantitative trait loci in *Arabidopsis thaliana*. *Proc. Natl Acad. Sci. USA* **107**, 21199–21204.
- Li, Y.H., Zhao, S.C., Ma, J.X. et al. (2013) Molecular footprints of domestication and improvement in soybean revealed by whole genome resequencing. *BMC Genom.* **14**, 579.
- Lipka, A.E., Tian, F., Wang, Q., Peiffer, J., Li, M., Bradbury, P.J., Gore, M.A., Buckler, E.S. and Zhang, Z. (2012) GAPIT: genome association and prediction integrated tool. *Bioinformatics*, **28**, 2397–2399.
- Lumsden, R.D. (1976) Pectolytic enzymes of *Sclerotinia sclerotiorum* and their localization in infected bean. *Can. J. Bot.* **54**, 2630–2641.
- Mamidi, S., Lee, R.K., Goos, J.R. and McClean, P.E. (2014) Genome-wide association studies identifies seven major regions responsible for iron deficiency chlorosis in soybean (*Glycine max*). *PLoS ONE*, **9**, e107469.
- Marciano, P., Di, L.P. and Magro, P. (1982) Polygalacturonase isoenzymes produced by *Sclerotinia sclerotiorum* in vivo and in vitro. *Physiol. Pl. Pathol.* **20**, 201–212.
- Morris, G.P., Ramu, P., Deshpande, S.P. et al. (2013) Population genomic and genome-wide association studies of agroclimatic traits in sorghum. *Proc. Natl Acad. Sci. USA* **110**, 453–458.
- Phipps, P.M. and Porter, D.M. (1982) Sclerotinia blight of soybean caused by *Sclerotinia minor* and *Sclerotinia sclerotiorum*. *Plant Dis.* **66**, 163–165.
- Pierantoni, L., Dondini, L., De Franceschi, P., Musacchi, S., Winkel, B.S.J. and Sansavini, S. (2010) Mapping of an anthocyanin-regulating MYB transcription factor and its expression in red and green pear, *Pyrus communis*. *Plant Physiol. Biochem.* **48**, 1020–1026.
- Rafalski, J.A. (2010) Association genetics in crop improvement. *Curr. Opin. Plant Biol.* **13**, 174–180.
- Salvatierra, A., Pimentel, P., Moya-Leon, M.A., Caligari, P.D.S. and Herrera, R. (2010) Comparison of transcriptional profiles of flavonoid genes and anthocyanin contents during fruit development of two botanical forms of *Fragaria chiloensis* ssp. *chiloensis*. *Phytochemistry*, **71**, 1839–1847.
- Schmutz, J., Cannon, S.B., Schlueter, J. et al. (2010) Genome sequence of the palaeopolyploid soybean. *Nature*, **463**, 178–183.
- Song, Q.J., Marek, L.F., Shoemaker, R.C., Lark, K.G., Concibido, V.C., Delannay, X., Specht, J.E. and Cregan, P.B. (2004) A new integrated genetic linkage map of the soybean. *Theor. Appl. Genet.* **109**, 122–128.
- Song, Q.J., Jia, G.F., Zhu, Y.L., Grant, D., Nelson, R.T., Hwang, E.Y., Hyten, D.L. and Cregan, P.B. (2010) Abundance of SSR motifs and development of candidate polymorphic SSR markers (BARCSOYSSR_1.0) in soybean. *Crop Sci.* **50**, 1950–1960.
- Sun, X., Liu, D., Zhang, X., Li, W., Liu, H., Hong, W., Jiang, C., Guan, N., Ma, C. and Zheng, H. (2013) SLAF-seq: an efficient method of large-scale de novo SNP discovery and genotyping using high-throughput sequencing. *PLoS ONE*, **8**(3), e58700.
- Vuong, T.D., Diers, B.W. and Hartman, G.L. (2008) Identification of QTL for resistance to sclerotinia stem rot in soybean plant introduction 194639. *Crop Sci.* **48**, 2209–2214.
- Wang, X., Zafian, P., Choudhary, M. and Lawton, M. (1996) The PR5K receptor protein kinase from *Arabidopsis thaliana* is structurally related to a family of plant defense proteins. *Proc. Natl Acad. Sci. USA* **93**, 2598–2602.
- Wang, S., Basten, C.J. and Zeng, Z.B. (2012) *Windows QTL Cartographer 2.5*. Raleigh, NC: Department of Statistics, North Carolina State University. (<http://statgen.ncsu.edu/qtlcart/WQTLCart.htm>).
- Wegulo, S.N., Yang, X.B. and Martinson, C.A. (1998) Soybean cultivar responses to *Sclerotinia sclerotiorum* in field and controlled environment studies. *Plant Dis.* **82**, 1264–1270.
- Wu, X., Ren, C., Joshi, T., Vuong, T., Xu, D. and Nguyen, H. (2010) SNP discovery by high-throughput sequencing in soybean. *BMC Genom.* **11**, 469.
- Yang, N., Lu, Y., Yang, X., Huang, J., Zhou, Y., Ali, F., Wen, W., Liu, J., Li, J. and Yan, J. (2014) Genome wide association studies using a new non-parametric model reveal the genetic architecture of 17 agronomic traits in an enlarged maize association panel. *PLoS Genet.* **10**, e1004573.
- Zhang, D., Song, H., Cheng, H., Hao, D., Wang, H., Kan, G., Jin, H. and Yu, D. (2014) The acid phosphatase-encoding gene GmACP1 contributes to soybean tolerance to low-phosphorus stress. *PLoS Genet.* **10**, e1004061.
- Zhao, K., Tung, C.-W., Eizenga, G.C. et al. (2011) Genome-wide association mapping reveals a rich genetic architecture of complex traits in *Oryza sativa*. *Nat. Commun.* **2**, 467.