**Arabidopsis GDSL1 overexpression enhances rapeseed Sclerotinia sclerotiorum resistance and the functional identification of its homolog in Brassica napus**

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**Summary**

Sclerotinia stem rot (SSR) caused by Sclerotinia sclerotiorum is a devastating disease of rapeseed (Brassica napus L.). To date, the genetic mechanisms of rapeseed interactions with S. sclerotiorum are not fully understood, and molecular-based breeding is still the most effective control strategy for this disease. Here, Arabidopsis thaliana GDSL1 was characterized as an extracellular GDSL lipase gene functioning in Sclerotinia resistance. Loss of AtGDSL1 function resulted in enhanced susceptibility to S. sclerotiorum. Conversely, overexpression of AtGDSL1 in B. napus enhanced resistance, which was associated with increased reactive oxygen species (ROS) and salicylic acid (SA) levels, and reduced jasmonic acid levels. In addition, AtGDSL1 can cause an increase in lipid precursor phosphatidic acid levels, which may lead to the activation of downstream ROS/SA defence-related pathways. However, the rapeseed BnGDSL1 with high sequence similarity to AtGDSL1 had no effect on SSR resistance. A candidate gene association study revealed that only one AtGDSL1 homolog from rapeseed, BnaC07g35650D (BnGLIP1), significantly contributed to resistance traits in a natural B. napus population, and the resistance function was also confirmed by a transient expression assay in tobacco leaves. Moreover, genomic analyses revealed that BnGLIP1 locus was not present in a selected region associated with SSR resistance during the breeding process, and its elite allele type belonged to a minor allele in the population. Thus, BnGLIP1 is the functional equivalent of AtGDSL1 and has a broad application in rapeseed S. sclerotiorum-resistance breeding.

**Introduction**

*Sclerotinia sclerotiorum* is a plant pathogen fungus that is notable for its wide host range and environmental persistence. *Sclerotinia* stem rot (SSR), caused by *S. sclerotiorum*, is a devastating disease of oil crops and has a worldwide distribution (Amselem et al., 2011; Rowe et al., 2010). Rapeseed (Brassica napus L.) is the third most important oil crop worldwide. In rapeseed farming, *S. sclerotiorum* causes the rotting of leaves, stems and pods, resulting in an annual 10%–20% yield loss in China, with up to 80% losses in severely infected fields (Oilcrop Research Institute and Chinese Academy of Sciences, 11975). *Sclerotinia sclerotiorum* also causes significant reductions in the oil yield, and the total chlorophyll, phenol and sugar contents of *Mentha arvensis* plants (Perveen et al., 2010). Currently, some new sources resistant to *S. sclerotiorum* have been screened and identified in *B. napus* and its wild relatives (Taylor et al., 2015, 2017; Uloth et al., 2013). However, it is difficult to use conventional breeding, based on phenotype, for SSR resistance in rapeseed because it is a complex quantitative trait. Therefore, molecular breeding is the most efficient and economic approach to control this disease in rapeseed. Using rapeseed with partial resistance, researchers have identified some quantitative trait loci for *S. sclerotiorum* resistance, which are valuable genetic resources for the molecular breeding of SSR resistance in rapeseed (Wei et al., 2014; Wu et al., 2013; Yin et al., 2010). At present, owing to the complex inheritance, as well as the paucity of resistant germplasms, there has been no report of an SSR resistance gene being cloned in rapeseed, and the genetic and molecular mechanisms for the variation in SSR resistance are still poorly understood.

In response to pathogen infection, plants can trigger various rapid and inducible defence responses, which function at different stages and to different degrees after pathogens contact the plant (Nomura et al., 2005; Schoonbeek et al., 2015; Stam et al., 2014; Talarczyk and Hennig, 2001). In plants with a resistant phenotype, the early recognition of pathogen effectors by the host resistance (R) proteins triggers a race-specific resistance, such as gene-for-gene resistance, which is often accompanied by the hypersensitive response (HR) and can provide a higher level of resistance against a few pathogen species (Ding et al., 2017; Hammond-Kosack and Jones, 1996; He et al., 2016; Kumar and Kirti, 2015). The roles of the phytohormones salicylic acid (SA) and jasmonic acid (JA) in the regulation of these inducible defences have been well established (Shen et al., 2017; Thaler et al., 2012). The signalling pathways activated by...
accumulations of these endogenous hormones regulate different defence responses that are effective against partially specific types of pathogens. For instance, resistance to pathogens with a biotrophic lifestyle is usually governed by SA-dependent signalling pathways, which generally induce the HR on infected sites, and induce programmed cell death (PCD) by blocking the nutrient sources of biotrophic pathogens, while JAVethylene (ET) -mediated defences contribute to the resistance against necrotrophic pathogens and herbivorous insects (Lee et al., 2015; Rahman et al., 2012; Zhang et al., 2010a). Coupled with SA signalling, reactive oxygen species (ROS) also participate in the formation of HR-related PCD, which defends against biotrophic pathogens (Yoshioka et al., 2009; Zhang et al., 2012). Crosstalk between signalling pathways is complex and is usually more important for the regulation of defence responses than signalling pathways themselves. SA-, JA- and ET-dependent signalling pathways act in both mutually synergistic and antagonistic manners during plant defence responses (Koornneef and Pieterse, 2008; Yang et al., 2015). Sclerotinia sclerotiorum is a hemi-biotrophic pathogen fungus that behaves like a biotroph without host cell necrosis during the early infection stages and then quickly converts to necrotrophic growth later (Kabbage et al., 2015). In contrast to the clearly elucidated resistance mechanisms to biotrophs or necrotrophs, there is still a lack of details regarding resistance to hemi-biotrophic pathogens.

GDSL-type lipases/esterases represent a subfamily of lipolytic enzymes in which the amino acid sequence contains a GDSL motif, GxSxxxxG, with the active site serine (S) located near the enzymes in animals and microorganisms, there is still a limited understanding of plant GDSL lipases, which were discovered relatively late. In recent years, plant GDSL lipases have received more attention because of their multifunctional properties, especially in relation to plant disease resistance and stress responses (Chepsyshko et al., 2012; Dong et al., 2016). In Arabidopsis, two GDSL LIpases (GLIP), GLIP1 and GLIP2, play key roles in resistance to Alternaria brassicicola and Erwinia carotovora, respectively (Lee et al., 2009; Oh et al., 2005). AtGLIP1 can modulate ET-associated systemic immunity through the regulation of ET signalling (Kim et al., 2013, 2014). The overexpression of A. thaliana Li-tolerant lipase 1 increases salt tolerance in yeast and Arabidopsis (Naranjo et al., 2006). In hot pepper (Capsicum annuum L.), GDSL lipase 1 may be involved in methyl jasmonic acid (MeJA) signalling pathways and/or contribute to wound-stress resistance by modulating C. annuum pathogenesis-related protein 4 (CaPR-4) expression (Kim et al., 2008). Another GDSL-type lipase gene isolated from hot pepper is GLIP1, which modulates disease susceptibility and abiotic stress tolerance in CaGLIP1 transgenic Arabidopsis (Hong et al., 2008). Two GDSL lipases, OsGLIP1 and OsGLIP2, negatively regulate defence responses by regulating lipid homeostasis in rice (Gao et al., 2017). Thus, GDSL lipases may function differently in plant–pathogen interactions across species. In the expression, the addition of TcGLIP is consistent with the pyrethrin content, natural insecticides, in Tanacetum cinerariifolium and is wound inducible, suggesting a role of plant GDSL lipases in their defence mechanisms against insects (Kikuta et al., 2012).

In a previous study, we found that two closely related GDSL motif-containing lipases, BnGDSL1 and AtGDSL1, were involved in regulating seed germination and seed oil content in B. napus (Ding et al., 2019). Here, based on their different functions in SSR resistance, we show that AtGDSL1, but not BnGDSL1, appears to be a critical component of the incompatible interaction with S. sclerotiorum. Moreover, the increased resistance to disease of AtGDSL1 transgenic rapeseed plants was correlated with enhanced phosphatidic acid (PA) production, which might, in turn, activate downstream ROS/SA defence signalling events that were critical for resistance. Further studies demonstrated that the counterpart of AtGDSL1 with respect to S. sclerotiorum resistance in B. napus was the BnGLIP1 gene harboured in selective regions on chromosome C07 during breeding. There was significant linkage among single-nucleotide polymorphisms (SNPs) within the BnGLIP1 gene, and the alleles harbouring SNPs exist in fewer varieties (36%) in the natural population, suggesting that BnGLIP1 could be used as a potential target to improve S. sclerotiorum resistance in rapeseed breeding.

**Results**

AtGDSL1 encodes a GDSL lipase localized in the extracellular space

We previously showed that AtGDSL1 belonged to the GDSL esterase/lipase family having the GDSL-motif (GDSXXXG) around the active site’s serine (Ding et al., 2019). To further confirm that AtGDSL1 encoded a GDSL lipase, we overexpressed AtGDSL1-fused GST in E. coli and investigated its lipase activity. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis analysis showed that the molecular mass of the AtGDSL1-GST protein was ~65 kDa, indicating that the AtGDSL1 gene could be expressed in E. coli. A Western blot analysis using a GST monoclonal antibody further confirmed the expression of the AtGDSL1 fusion protein (Figure S1a). The lipase activity of the purified recombinant AtGDSL1 fusion protein was then assayed using two p-nitrophenyl derivatives with different acyl group chain lengths, p-nitrophenyl laurate (C12) and p-nitrophenyl palmitate (C16). As shown in Figure S1b, increased product concentration was detected with both substrates. The enzymatic product increased gradually over 1 h, and the final activity reached 7.2 mM/mg for C12 and 4.24 mM/mg for C16 at 5 h. Thus, the AtGDSL1 protein was verified to be a lipase with lipase activity.

To investigate the subcellular localization of AtGDSL1, an enhanced green fluorescent protein (eGFP) was fused at the C terminus of AtGDSL1 (35S::AtGDSL1-eGFP). Plants that transiently expressed GFP driven by the 35S promoter (35S::eGFP) served as positive controls (Figure 1a). Fluorescence from GFP was initially examined in lower leaf epidermis by laser scanning confocal microscopy. The fluorescence signal of AtGDSL1 was clearly detected between the cells, indicating that AtGDSL1 may localize at the extracellular space, cell wall or cell membrane (Figure 1b, upper). To further locate AtGDSL1, protoplasts were prepared from leaves that transiently expressed AtGDSL1-eGFP. As shown in Figure 1b (lower), the eGFP signal disappeared, indicating that AtGDSL1 did not localize to the cell membrane. Combined with the bioinformatics analyses that AtGDSL1 may be a secreted protein with the signal peptide sequence at the N terminus, we concluded that AtGDSL1 might be an extracellular protein that is secreted into the cell wall or extracellular space. 

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The insertional mutations of \textit{AtGDSL1} in \textit{Arabidopsis} enhanced susceptibility to \textit{S. sclerotiorum}

To explore the function of \textit{AtGDSL1} in \textit{Arabidopsis}' response to \textit{S. sclerotiorum} inoculation, three T-DNA insertion mutants (SALK_025240C, CS352665 and CS352839) of \textit{AtGDSL1} were used. qRT-PCR analysis indicated that no \textit{AtGDSL1} transcripts were detected in its homozygous mutants (Figure 2a). The phenotype assays showed that the mutants had no obvious morphological differences compared with wild-type (WT). Then, the resistance phenotypes were investigated. As shown in Figure 2b (upper), disease symptoms were noted at 24 h after inoculation in both mutants and WT. However, the insertion mutants showed a significantly increased severity, with the rapid spreading of necrotic lesions after inoculation. The average lesion area in the \textit{AtGDSL1} insertion mutants was nearly three times greater than in WT (Figure 2b, lower). Thus, the knockout of \textit{AtGDSL1} in \textit{Arabidopsis} increased the susceptibility to \textit{S. sclerotiorum} infection. Phylogenetic analysis of GDSL motif-containing lipases/hydrolases from plants revealed that \textit{AtGDSL1} was most closely related to \textit{BnGDSL1} (BnaC03g59270D), with 86% similarity in their amino acid sequences (Ding et al., 2019). Three independent RNAi lines (\#10, \#13 and \#17) expressing a \textit{BnGDSL1}-specific sequence were tested for their resistance to \textit{S. sclerotiorum}. However, leaves from \textit{BnGDSL1}-RNAi plants showed no significant differences in symptoms or lesion sizes compared with control plants (Figure 2c).

Overexpression of \textit{AtGDSL1} in \textit{B. napus} enhanced resistance to \textit{S. sclerotiorum}

The transient overexpression of \textit{AtGDSL1} and \textit{BnGDSL1} in \textit{Nicotiana benthamiana} was performed to determine whether they are required for basal resistance against \textit{S. sclerotiorum}. Compared with WT, the inoculation of leaves transiently expressing \textit{AtGDSL1} with \textit{S. sclerotiorum} resulted in a significant inhibitory effect on disease symptoms, and the disease’s spot size was reduced nearly threefold, while the inoculation of leaves transiently expressing \textit{BnGDSL1} with \textit{S. sclerotiorum} had no effect (Figure S2).

To further address the involvement of \textit{AtGDSL1} in plant defence against \textit{S. sclerotiorum}, homozygous \textit{AtGDSL1} overexpression (OE) lines (\#12, \#18 and \#35) and \textit{BnGDSL1}-OE lines (\#22, \#28 and \#36) were selected from the positive transgenic rapeseed plants to evaluate their resistance to \textit{S. sclerotiorum} (Ding et al., 2019). The phenotype analysis demonstrated that \textit{AtGDSL1}-OE lines showed much smaller chlorotic/necrotic lesions relative to the control plants, while the symptoms on leaves from \textit{BnGDSL1}-OE plants were more severe but not different than the control plants (Figure 3a). This result was consistent with the \textit{S. sclerotiorum} inoculation phenomenon in \textit{N. benthamiana} leaves. Moreover, disease development was investigated at different periods of the infection process. As shown in Figure 3b and Figure S3, the soft-rotting necrosis occurred as early as 24 h after inoculation. The lesion size in \textit{AtGDSL1}-OE lines was significantly smaller than in WT. The differences became apparent at 36 h and reached a maximum
AtGDSL1 expression was strongly induced in AtGDSL1 transgenic plants, indicating that ROS accumulation strongly stained before and after infection (Figure 4a). Furthermore, the hyphal growth of S. sclerotiorum in the infected leaves was also examined by trypan blue staining. Mycelial growth was tightly aggregated and restricted to the necrotic zone in overexpressing AtGDSL1 leaves, while it loosely spread beyond this zone in WT and BnGDSL1 leaves (Figure 3c). Compared with WT plants, basal levels of lipase activity were much greater in the AtGDSL1 and BnGDSL1 transgenic plants. Moreover, the levels of lipase activity were more strongly induced in AtGDSL1 transgenic plants after S. sclerotiorum infection (Figure 3d). Thus, the lipase activity of AtGDSL1 may be closely related to plant defence responses against S. sclerotiorum. In addition, we carried out stem inoculations at the flowering stage, and the increased resistance levels in the lines overexpressing AtGDSL1 were also confirmed (Figure 3e). These results suggest that AtGDSL1 was a critical factor in S. sclerotiorum resistance and the overexpression of this gene in rapeseed increased disease resistance. 

S. sclerotiorum infection induced ROS accumulation in AtGDSL1 transgenic plants

To explore the molecular mechanisms of AtGDSL1-regulated resistance in rapeseed, we first used 3,3′-diaminobenzidine (DAB) staining to detect the accumulation of hydrogen peroxide (H₂O₂), a relatively stable ROS. Compared with WT rapeseed, the leaves of AtGDSL1 transgenic plants were more strongly stained before and after infection (Figure 4a). Furthermore, the endogenous ROS level was much higher in AtGDSL1 transgenic plants, indicating that ROS accumulation was strongly induced in AtGDSL1 transgenic plants during the early stages of infection (Figure 4b). We further examined the expression of NADPH oxidase and polyamine oxidase (PAO), which are marker genes associated with H₂O₂ production in plants (Ding et al., 2011; Keller et al., 1998; Yoda et al., 2006). The transcripts of those genes were significantly induced in transgenic rapeseed plants following S. sclerotiorum infection. In particular, the expression of NADPH oxidase increased rapidly and peaked at 12 h, while PAO gradually increased within 24 h following inoculation (Figure 4c). These data suggest that the overexpression of AtGDSL1 promoted ROS accumulation. Moreover, the activities of the ROS-scavenging enzymes, superoxide dismutase (SOD) and peroxidase (POD), were significantly reduced in the leaves of AtGDSL1-OE plants, compared with WT control plants, which was consistent with the accumulation of ROS (Figure S4a).

AtGDSL1 regulates Sclerotinia resistance in rapeseed by modulating SA- and JA-dependent pathways

To determine whether AtGDSL1’s regulation of S. sclerotiorum resistance in rapeseed involves SA and JA defence-related signalling pathways, the time-course expression profiles of gene sets associated with SA and JA pathways were examined in samples collected from infected leaf tissues of the WT and the resistant AtGDSL1-OE rapeseed plants. In Arabidopsis, the isochorismate pathway is generally responsible for SA synthesis (Wildermuth et al., 2001). The expression of the rapeseed homolog of Arabidopsis’ isochorismate synthase 1 (ICS1) showed no significant change during S. sclerotiorum infection (Figure 5a). However, the expression of phenylalanine ammonia lyase (PAL) gene, a key enzyme in SA biosynthesis through the phenylpropanoid pathway (Lee et al., 1995), was significantly induced in AtGDSL1-OE plants, peaking at 18 h. Similar expression patterns were also observed for PR2, which is a marker gene for SA-mediated defence responses, except that it showed a
much more efficient induction at 24 h in AtGDSL1-OE plants. The homolog of non-expressor of PR genes 1 (NPR1), a positive regulatory protein of SA-induced systemic acquired resistance, exhibited an induction trend only in AtGDSL1-OE plants, with the transcripts peaking at ~18 h (Figure 5a). The genes investigated for JA signalling included the JA synthesis gene 3-keto-acyl-CoA thiolase 4 (KAT4) as well as the JA responsive genes lipoxygenase 2 (LOX2) and PR3. The expression levels of these genes were all repressed in AtGDSL1 transgenic plants (Figure 5b). The enhancement of SA signalling and attenuation of JA signalling could be critical factors in S. sclerotiorum resistance in AtGDSL1-OE plants.

To further investigate whether AtGDSL1 affects SA and JA accumulation during S. sclerotiorum’s response to infection, their endogenous contents were measured in WT, AtGDSL1-OE and insertion lines at different time points following infection. The variation in SA and JA contents were consistent with the expression profiles of the investigated genes in the respective pathways. Compared with WT, AtGDSL1 overexpression plants showed a tendency to accumulate more SA during the first 24 h after infection (Figure 5c). In contrast, the changes in JA levels in transgenic plants were much less prominent relative to those in WT (Figure 5d). The constitutive expression of AtGDSL1 also

**Figure 3** Transformation of rapeseed with AtGDSL1 and BnGDSL1 and the disease symptoms of transgenic plants inoculated with S. sclerotiorum. (a) Phenotypes of WT and T2 transgenic plants (BnGDSL1 T28 and AtGDSL1 T18) 36 h after inoculation with S. sclerotiorum. Bars = 1.0 cm. (b) Leaf lesion area measurements from 24 to 60 h after S. sclerotiorum infection. Differences in susceptibility between WT and AtGDSL1 transgenic lines were significant (P < 0.001) from 36 to 60 h. Data are means ± SDs from three independent experiments, each with 15 leaves. (c) The growth of S. sclerotiorum examined by trypan blue staining. a1, a2, a3 and a4: S. sclerotiorum-infected WT and transgenic leaves. Photographs were taken at 20 h post-inoculation; b1, b2, b3 and b4: Inoculated leaves stained with trypan blue. Photographs were taken at 4 h after staining; c1, c2, c3 and c4: mycelial growth on stained leaves, visualized using a fluorescence microscope. Bars indicate 1.0 cm in a1-a4 and b1-b4 panels and 1 mm in c1-c4 panels. (d) Lipase activity assay in leaves of ‘NY12’ and AtGDSL1/BnGDSL1-OE plants after 24 h of S. sclerotiorum treatment. Data are means ± SD from three independent experiments. The significant differences between treated and untreated (control) samples in each line are indicated (Student’s t-test: *, P < 0.05; **, P < 0.01). (e) Lesion phenotypes (upper) and lesion lengths (down) in WT and T2 transgenic plant stems 7 days after inoculation with S. sclerotiorum. Bars = 1.0 cm. Significant differences between WT and transgenic plants are indicated (Student’s t-test: *, P < 0.05). The experiment was repeated three times with similar results.

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affected the contents of other oxylipins, such as 12-oxo-phytodienoic acid (12-OPDA) and jasmonoyl-L-isoleucine (JA-Ile), which changed in a manner similar to JA across the time points (Figure S5). Moreover, the basal levels of SA and JA were also affected in transgenic plants, which exhibited a relatively higher level of SA and lower level of JA than WT plants. Conversely, \textit{AtGDSL1} insertion mutants increased the accumulation of JA and decreased SA levels (Figure 5e). Thus, \textit{AtGDSL1} overexpression in rapeseed can effectively increase SA accumulation and reduce JA accumulation in response to \textit{S. sclerotiorum} infection, which are likely associated with enhanced resistance.

\textit{AtGDSL1} positively regulates phosphatidic acid (PA) levels, leading to the activation of downstream defence pathways

To further investigate whether the altered resistance in \textit{AtGDSL1} plants was due to the high SA or low JA levels, we investigated the effects of exogenous SA and JA in transgenic plants, which exhibited a relatively higher level of SA and lower level of JA than WT plants. Conversely, \textit{AtGDSL1} insertion mutants increased the accumulation of JA and decreased SA levels (Figure 5e). Thus, \textit{AtGDSL1} overexpression in rapeseed can effectively increase SA accumulation and reduce JA accumulation in response to \textit{S. sclerotiorum} infection, which are likely associated with enhanced resistance.

\textit{AtGDSL1} overexpression increased ROS accumulation. (a) DAB staining in the leaves of ‘NY12’ and \textit{AtGDSL1-OE} plants after treatment for 24 h with \textit{H₂O₂} or \textit{S. sclerotiorum}. Bars = 400 μm. (b) Quantification of ROS levels in leaves of ‘NY12’ and \textit{AtGDSL1-OE} plants after 24 h of treatment. (b) Expression profiles of \textit{NADPH oxidase} and \textit{PAO} in ‘NY12’ and \textit{AtGDSL1} transgenic rapeseed plants after \textit{S. sclerotiorum} infection. The relative expression levels were analysed by qRT-PCR and normalized using \textit{BnTIP41} as the internal control. The experiment was repeated three times with similar results. Data represent the means ± SD from three independent experiments. Significant differences between \textit{AtGDSL1} transgenic lines and WT at each time point are indicated (Student’s t-test) as follows: ***, \textit{P} < 0.001; **, \textit{P} < 0.01; *, \textit{P} < 0.05.

To explore how an apoplastic lipase affected the SA/ROS pathway, we further monitored the content of PA, which is the precursor for the biosynthesis of many other lipids and acts as an important lipid secondary messenger in cell growth and stress responses. The \textit{AtGDSL1} overexpression caused an increase in PA levels, with a pattern similar to those of SA/ROS-related genes (Figure 6e). Also, PA levels were decreased in the \textit{AtGDSL1} knockout mutants (Figure 6f).

Identification of the functional homolog of \textit{AtGDSL1} in \textit{B. napus}

Based on the different \textit{S. sclerotiorum} resistance phenotypes of plants overexpressing \textit{AtGDSL1} and \textit{BnGDSL1}, we inferred that \textit{BnGDSL1} was not the counterpart of \textit{AtGDSL1} in this respect, even though their sequences were similar. To validate the function of \textit{BnGDSL1} and identify the functional homolog of \textit{AtGDSL1} in \textit{B. napus}, we performed a candidate gene association analysis within a set of 324 rapeseed accessions having different resistance levels. Finally, 926 SNPs in 33 highly homologous genes...
were selected for an association analysis using disease index and disease rate. The most significantly associated gene among the homologs was the BnaC07g35650D locus, designated BnGLIP1, in which 15 significant SNPs explain 7.40%–10.49% of the disease index variation and 14 SNPs explain 6.80%–9.13% of the disease rate variation in this population (Figure 7a, Table 1). We also analysed the association between the SNPs and the resistance phenotype. Five significantly associated SNPs (GDSL_37986315, 7987132, 7987173, 37987342 and 37987371) were in exon sequences of BnGLIP1, and the corresponding lines containing these SNPs exhibited greater resistance to S. sclerotiorum (Table 1). The QQ plot was in good agreement with the Manhattan plot when estimating the associations of SNPs with the two resistance traits (Figure 7b).

An expression analysis revealed that BnGLIP1 transcripts were significantly induced by S. sclerotiorum infection in the resistant cultivar Zhongshuang 11 (Figure 8a). In addition, BnGLIP1 was inducible by SA but was not increased by applications of JA or 1-aminocyclopropane-1-carboxylic acid (ACC) (Figure 8b). The full-length cDNA of BnGLIP1 was then cloned from Zhongshuang 11 (Figure S6). It also encodes a GDSL-like lipase protein with conserved GDSL motifs, catalytic sites and ATP/GTP-binding sites, and is homologous with AtGDSL1, sharing a 68% sequence identity (Figure S7). The transient overexpression of BnGLIP1 in N. benthamiana showed a significantly reduced disease severity, with chlorosis and necrosis confined to the inoculation sites, which was very similar to AtGDSL1 (Figures 8c). Thus, BnGLIP1 may be the functional counterpart of AtGDSL1 with respect to S. sclerotiorum resistance in B. napus.

BnGLIP1 is harboured in the selective regions during rapeseed breeding

To examine whether the BnGLIP1 gene had experienced selection during rapeseed breeding, we detected the genomic regions with strong selective sweep signals on chromosome C07 (Data S1). By comparing the level of genetic diversity (\( \pi \)) between the resistant (R) and susceptible (S) subgroups, we detected two strong selection signals in the regions of the S subgroup harbouring the BnGLIP1 locus, in which the \( \pi \) ratios (\( \pi_R/\pi_S \)) were 1.60 and 1.50 for the disease index (Figure 9a) and 1.55 and 1.62 for the disease rate (Figure 9b), respectively. The selective sweeps located around the BnGLIP1 locus were also confirmed by calculating the

Figure 5  AtGDSL1 affected SA- and JA-mediated signalling pathways. (a) AtGDSL1 positively regulated SA-related genes PAL, PR2 and NPR1. (b) AtGDSL1 negatively regulated JA-related genes KAT4, LOX2 and PR3. The relative expression levels were analysed by qRT-PCR and normalized using TIP41 as an internal control. Values are means ± SDs from three replicates. (c and d) AtGDSL1 affects endogenous SA (c) and JA (d) levels in ‘NY12’ and transgenic rapeseed plants after the infection. (e) Quantification of SA and JA contents in WT and AtGDSL1 insertion mutant lines. Data are means ± SDs from three independent experiments. The significant differences between ‘NY12’ and AtGDSL1-OE at each time point, or between Col-0 and AtGDSL1 insertion lines, are indicated (Student’s t-test) as follows: ***, \( P < 0.001; **, P < 0.01; *, P < 0.05)."
population differentiation index (FST) between the two subgroups (FST values were 0.096 and 0.104 in Figure 9a and 0.117 and 0.105 in Figure 9b). Moreover, we found that more than 85% of the selective sweeps identified using the \( \rho \) ratio approach could also be identified using the FST values, indicating that the identified selective regions were quite reliable. Thus, the BnGLIP1 gene appears to have experienced selection during rapeseed breeding, which further confirmed the functional importance of BnGLIP1 responsible for SSR resistance.

**Allelic variation analysis of BnGLIP1**

We further studied the linkage disequilibrium (LD) of SNPs in the BnGLIP1 locus and found that they exhibited significant linkage, especially for the first 15 SNPs (Figure 10a). The SNP GDSL_chrC07_37987173, which was located on exon 3 and showed the greatest contributions to disease index and disease rate, was selected for the investigation of the BnGLIP1 allelic distribution in the population. The SNPs formed three allelic types (CC, CT and TT) associated with significantly different resistance traits. The average disease index and disease rate were 42.32% and 49.34%, respectively, when the SNP was CC \((n = 113)\), 48.58% and 51.00%, respectively, for CT \((n = 12)\), and 59.38% and 64.84% for TT \((n = 191)\), suggesting that the best allele type was CC (Figure 10b, Data S2). Most of the rapeseed accessions (60%) carried the TT type, while only 113 accessions (36%) carried the CC type, indicating that the BnGLIP1 locus still has great potential in improving S. sclerotiorum resistance.

**Discussion**

AtGDSL1 functions differently than BnGDSL1 in increasing S. sclerotiorum resistance in rapeseed

In Arabidopsis and other plants, loss-of-function and gain-of-function studies have demonstrated that the GDSL lipases are involved in many important biological processes in plants, such as environmental stress response, seed development, pathogen defence and lipid metabolism (Chepyshko et al., 2012; Clauss et al., 2011; Rombolá-Caldentey et al., 2014; Takahashi et al., 2010), but little is known regarding specific GDSL lipases’ roles in the defence against the pathogen S. sclerotiorum in B. napus. Our study showed several lines of evidence suggesting that AtGDSL1 participates in the defence response against S. sclerotiorum. First, suppressing AtGDSL1 mediated by T-DNA insertions in Arabidopsis led to an increased susceptibility to S. sclerotiorum infection (Figure 2). Second, the enhanced resistance to the expansion of S. sclerotiorum was validated in rapeseed and N. benthamiana transgenic plants that expressed AtGDSL1 (Figures 3, Figure S1). Finally, the extracellular localization of AtGDSL1 (Figure 1b) led us to infer that it may be associated with a disease-resistance function in vivo because the infection...
can be more effectively suppressed before the pathogen enters the cell (Del Rio et al., 2017; Selitrennikoff, 2001). As reported previously, AtGLIP1, which is a homolog of GDSL1 in Arabidopsis and is also secreted into the cell wall or extracellular space, could defend against A. brassicicola infection by directly disrupting fungal spore integrity. This function of GLIP1 is dependent on its lipase activity (Oh et al., 2005). Because AtGDSL1 is also a secreted protein and AtGDSL1 transgenic plants exhibit higher lipase activity levels than WT after infection (Figure 3d), it is conceivable that AtGDSL1 may have the ability to attack the invading S. sclerotiorum. In addition, there has been some debate on stem resistance vs. leaf resistance (Uloth et al., 2013; Taylor et al., 2017). Some research suggests that they are correlated, while other research suggests that they are genetically distinct traits. In our study, the increased resistance in AtGDSL1-OE lines was confirmed using stem inoculations (Figures 3e).

In our study, all the transgenic materials were selected according to the 3:1 segregation of antibiotic resistance. Therefore, the overexpression of AtGDSL1 and BnGDSL1 in plants resulted from single copy insertions, indicating that the transcriptional levels of AtGDSL1 and BnGDSL1 did not vary too much. Although BnGDSL1 could be induced by S. sclerotiorum infection and is responsive to SA and JA treatments (Figure S8), the overexpression of BnGDSL1 in N. benthamiana and B. napus did not lead to a statistically significant difference in lesion size or lesion spread rate compared with the recipient parents (Figures 3, Figure S1). Thus, it appears that BnGDSL1 and AtGDSL1 function differently in increasing resistance to S. sclerotiorum in B. napus.

AtGDSL1 may contribute to the defence against S. sclerotiorum in association with PA-ROS/SA signalling pathways

The role of lipids and lipases in cellular defence signalling has been reported previously (Falk et al., 1999; Jirage et al., 1999; Maldonado et al., 2002). Because AtGDSL1 is an apoplastic lipase, it may function in the generation of a lipid-derived molecule through lipid-hydrolyzing activity upon pathogen challenge. In accordance with this hypothesis, increased PA levels were detected in OE plants. PA, as the most important phospholipid signalling molecule, is implicated in modulating oxidative bursts and hormone signalling in plants (Chen et al., 2019).
Immunity-stimulated PA production is required for SA-dependent defence activation and exhibits a biphasic pattern that precedes ROS generation and SA accumulation, leading to downstream defence responses (Zhang and Xiao, 2015). Thus, the AtGDSL1-induced activation of PA signalling may be a relatively early defence event, and further monitoring of the concentrations and spatial-temporal changes of PA at the subcellular level when challenged with S. sclerotiorum can better reveal resistance mechanisms.

In AtGDSL1-OE plants, SSR resistance is associated with ROS burst, which is caused by the up-regulation of NADPH oxidase and PAO (Figure 4b). The accumulation of ROS in the early stages of infection could behave as a signal to induce SA signalling (Grant and Lamb, 2006). The accumulation of ROS could also participate in the formation of HR-related PCD coupled with SA signalling, which results in the appearance of lesions to inhibit S. sclerotiorum growth during the short biotrophic phase. In plants, two independent pathways, including those catalysed by ICS1 and PAL, are involved in SA biosynthesis. In our study, only PAL was significantly induced in AtGDSL1 transgenic plants. The expression of NPR1 also showed a similar expression pattern to PAL in AtGDSL1 transgenic plants (Figure 5a). Thus, AtGDSL1 can directly or indirectly affect SA biosynthesis mainly through the PAL pathway, and alterations in the SA levels subsequently cause variations in NPR1 expression (Li et al., 2013b; Zhang et al., 2010b). The requirement for PAL-dependent SA accumulation in resistance is also supported by other pathosystems, including those based on Arabidopsis–Peronospora parasitica, soybean–Phytophthora sojae and wheat–Fusarium graminearum, in which

| SNPs             | Position | Disease_index | Disease_rate | Genotype | R  | S  |
|------------------|----------|---------------|--------------|----------|----|----|
| chrC07_37986315  | 3'-UTR   | 9.54          | 8.09         | G        | C  |    |
| chrC07_37987132  | exon     | 8.00          | 7.07         | T        | C  |    |
| chrC07_37987173  | exon     | 10.49         | 9.13         | G        | A  |    |
| chrC07_37987342  | exon     | 8.54          | 7.84         | G        | T  |    |
| chrC07_37987371  | exon     | 8.18          | 7.22         | G        | C  |    |
| chrC07_37987575  | exon     | 7.39          | ns           | C        | A  |    |
| chrC07_37988334  | 5'-UTR   | 9.81          | 8.41         | G        | T  |    |
| chrC07_37988360  | 5'-UTR   | 9.21          | 7.95         | A        | G  |    |
| chrC07_37988367  | 5'-UTR   | 8.97          | 8.45         | G        | A  |    |
| chrC07_37988549  | 5'-UTR   | 8.67          | 8.74         | G        | T  |    |
| chrC07_37988692  | 5'-UTR   | 7.85          | 6.81         | A        | A/W|    |
| chrC07_37988891  | 5'-UTR   | 8.97          | 7.02         | R        | T  |    |
| chrC07_37988988  | 5'-UTR   | 8.99          | 8.34         | G        | M/A|    |
| chrC07_37989153  | 5'-UTR   | 8.76          | 8.49         | M/A      | G  |    |
| chrC07_37989463  | 5'-UTR   | 8.65          | 7.00         | T        | C  |    |

R and S indicate extremely resistant and susceptible materials, respectively; ns: no significant.

Table 1 Fifteen significantly associated SNPs detected in BnaC07g35650D locus

Figure 8 BnGLIP1 confers resistance to S. sclerotiorum in N. benthamiana. (a–b) Time-course expression analyses of BnGLIP1 after S. sclerotiorum infection (a) and phytohormone (ACC, MeJA and SA) treatment (b) in rapeseed. The expression levels at 0 h (no treatment) were quantified by qPCR and served as the control. The data represent the means ± SDs from three independent experiments. The significant differences in gene expression levels between each time point and the control are indicated (Student’s t-test) as follows: ***, P < 0.001; **, P < 0.01; *, P < 0.05. (c) Disease-resistance effects of transient expression of AtGDSL1 or BnGLIP1 in N. benthamiana. Photographs were taken at 24 h post-inoculation with S. sclerotiorum on leaves inoculated with A. tumefaciens carrying 35S::00, 35S::AtGDSL1 or 35S::BnGLIP1. Bars = 1.0 cm. The data represent the means ± SDs from three independent experiments, with each containing 15 leaves. Significant differences in lesion size between transgenic lines and WT are indicated (Student’s t-test) as follows: ***, P < 0.001; **, P < 0.01; *, P < 0.05.
mutants or RNAi lines deficient in the SA-response pathway exhibited reduced SA marker gene expression levels and enhanced susceptibility to pathogens (Ding et al., 2011; Mauch-Mani and Slusarenko, 1996; Zhang et al., 2017). Moreover, the expression of NADPH oxidase and PAO is similar to those of SA-response genes, which is not surprising since ROS bursts can stimulate SA accumulation and, in turn, SA can induce ROS production through a positive feedback loop (Chen et al., 1993; Leon et al., 1995).

Based on the present results, we propose a model to illustrate the role of AtGDSL1 in plant early immune responses when challenged with S. sclerotiorum (Figure S9). Attacks on transgenic rapeseed plants by S. sclerotiorum can release PA from plasma membranes by the lipid-hydrolyzing activity of AtGDSL1, which positively activates membrane localized NADPH oxidase and stimulates ROS production. ROS production also contributes to the activation of SA signalling, and both events are related to HR/PCD. Moreover, there was a close association among these defence-related signalling events. The enhanced SA signalling induces the expression of AtGDSL1 and downstream genes in SA pathways. AtGDSL1 causes more SA accumulation by regulating the biosynthetic gene PAL. As a result, the positive feedback arising between the SA accumulation and the induction of AtGDSL1 may lead to the amplification of the SA defence pathway, which has a negative effect on the JA-dependent pathway. Thus, the AtGDSL1-regulated enhancement of SA signalling and attenuation of JA signalling may be important for the occurrence of early resistance or defence reactions. Further investigation of the effective concentration range of these signalling molecules may better reveal the defence mechanisms and increase the practical value in breeding rapeseed.

**BnGLIP1** is a promising target for increasing S. sclerotiorum resistance in rapeseed breeding

Candidate gene association analyses based on natural populations are very effective in discovering alleles and functional genes that make large contributions to a target trait. Using this method, we found that another GDSL1 homolog (BnGLIP1) in Brassica may be the main functional gene in SSR resistance. Increasing crop resistance is a selective process, and multiple loci and their genomic regions have experienced selection throughout the history of plant breeding. Thus, the identification of selective regions and their imbedded functional genes or loci could benefit future plant breeding. We found that the BnGLIP1 gene was located within the selective region of the S subgroup (Figure 9), which enabled us to confirm the BnGLIP1 locus' contribution to the SSR-resistance trait during rapeseed breeding. According to the genetic diversity analysis, BnGLIP1 showed a greater polymorphism level in the R subgroup, suggesting that some susceptibility loci might still exist in areas of resistant varieties. These loci can be identified and modified to increase the resistance levels of these moderately

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**Figure 9** Selective sweep signals on chromosome C07 in resistant (R) and susceptible (S) rapeseed subgroups. (a) Comparisons of disease index between the R and S subgroups. (b) Comparisons of disease rates between the R and S subgroups. The \( \pi \) ratios (\( \pi_R/\pi_S \)) and \( F_{ST} \) values were calculated in 20 kb sliding windows with a step size of 2 kb. The vertical red lines correspond to the 5% left and right tails of the empirical \( \pi \) ratio distribution, where the \( \pi \) ratios are 0.52 and 1.47 for the disease index (a), and 0.49 and 1.51 for the disease rate (b) between R and S subgroups, respectively. The horizontal red lines correspond to the 5% right tail of the empirical \( F_{ST} \) distribution, where \( F_{ST} \) is 0.087 in (a) and 0.083 in (b). The data points located to the left and right of the vertical red lines, and above the horizontal red line, are the selective sweeps related to the disease index and disease rate for the R (blue dots) and S (green dots) groups, respectively. Red dots indicate regions including the BnGLIP1 gene.
resistant varieties. Therefore, the \textit{BnGLIP1} gene has potential applications and prospects in rapeseed resistance breeding.

Generally, SNPs in the coding sequences of candidate genes are considered important for successful molecular breeding (Zhang et al., 2018). After excluding some SNPs in non-coding sequences and three synonymous substitutions, we suggest that two SNPs, 361C/G and 484C/T, in the exons of \textit{BnGLIP1}, causing nonsynonymous substitutions, may be functional SNPs that affect SSR resistance (Figure S6). An allelic variation analysis revealed that only a small percentage of rapeseed accessions contained the resistance-related minor allele type (Figure 10), suggesting that most varieties can have increased \textit{S. sclerotiorum} resistance by the introgression of the resistance-related allele during the breeding process. In addition, the amino acid sequence of \textit{BnGLIP1} also contained predicted signatures (GRFSNGKT) for an ATP/GTP-binding site (P-loop), which is found in most disease R genes in plants, such as Rx, N, RPS2 and RPM1. Amino acid 71K, located in the P-loop motif, is important for nucleotide binding site and leucine-rich repeats R genes. A substitution at this residue can lead to the resistance proteins losing their functions (Tameling et al., 2002). However, whether the difference in function between \textit{BnGDSL1} and \textit{BnGLIP1} is the result of a single base difference in the P-loop motif remains to be examined.

The study of \textit{AtGDSL1}-regulated \textit{S. sclerotiorum} resistance in \textit{Brassica} provides us with some important clues for understanding the interaction mechanisms between host and \textit{Sclerotinia}. The further exploiting of the \textit{BnGLIP1} gene's functions, as well as verifying the candidates of functional SNPs in \textit{BnGLIP1} using genetic approaches like overexpression or the CRISPR/Cas9 system, will aid in the development of effective molecular markers in \textit{B. napus} and increase its practical value in breeding rapeseed with \textit{S. sclerotiorum} resistance.

**Experimental procedures**

**Plant materials and growth conditions**

The seeds of WT rapeseed cultivars 'NY12' and 'Zhonghuang 11', \textit{N. benthamiana} and \textit{Arabidopsis} wild-type (Col-0) stored in our laboratory were sown in flower pots (4–5 seedlings/pot) and grown in a plant growth room following growth conditions described by Ding et al. (2019). We searched the \textit{Arabidopsis} Biological Resource Centre at Ohio State University (http://www.arabidopsis.org/abrc/) and obtained three T-DNA insertion mutants, SALK 025240C, CS352665 and CS352839, in which the insertion sites were all located in the exon regions of the \textit{AtGDSL1} gene.

**Chemical treatments and \textit{S. sclerotiorum} infection**

Four-leaf-stage rapeseed seedlings were sprayed with 1 mM SA, 100 \textmu M ACC and 100 \textmu M MeJA (all from Sigma, St. Louis, MO) independently, and were sampled at 0, 3, 6, 9, 12 and 24 h. Each treatment was repeated three times.

For \textit{S. sclerotiorum} treatments, the fungal strains preserved at 4 °C were subcultured on potato dextrose agar medium first. Then, the new marginal hyphae were excised using a 7-mm puncher and were closely upended onto the adaxial surface of leaves from four-leaf-stage rapeseed seedlings. The inoculated plants were placed in a humidification chamber and covered with saran wrap to maintain moisture that allowed for the development of disease symptoms. The infected leaves were harvested at 6, 12, 18, 24, 36 and 48 h following inoculation. The field stem inoculations with mycelial agar discs were carried out at the flowering stage as described in Wang et al. (2018c). Three biological replicates were performed for each sample in this experiment.
RNA extraction, cDNA synthesis and quantitative RT-PCR (qRT-PCR)

RNA extractions, reverse transcription and qRT-PCR were performed according to Wang et al. (2018a, 2018b). The relative expression levels were estimated using the 2–ΔΔCT method of Livak and Schmittgen (2001). The PCR was repeated with three biological replications. Primers and annealing temperature used for RT-PCR and qPCR are listed in Table S1.

Protein expression, purification and Western blotting

A recombinant plasmid expressing AtGDSL1 was constructed by cloning the coding region of AtGDSL1 using the ATEcoRI-F and ATEcoRI-R primers. The PCR products were digested with EcoRI and Xhol and inserted into a pGEX-4T-1 vector (Novagen, San Diego, CA). The recombinant plasmid was then introduced into the Escherichia coli strain Transetta (DE3) (TransGen Biotech, Beijing, China) as described by Xiang et al. (2015). E. coli transformed with the empty vector served as a control. The recombinant protein was purified using metal chelate chromatography with Protein Iso DST Resin (TRAN, Beijing, China) according to the manufacturer’s protocol. SDS-PAGE and Western blotting were carried out according to Tan et al. (2014).

Agrobacterium-mediated transient expression in N. benthamiana leaves and confocal fluorescence microscopy analysis

To create a C-terminal fusion of AtGDSL1 with eGFP, the full-length cDNA of AtGDSL1 was PCR amplified using the AtGDSL1-SLR primers. The resulting fragment was cloned into the pENTR vector. It was then subcloned into the destination vector pK7FWG2.0 using Gateway LR recombinase (Invitrogen, Carlsbad, CA). The plasmids, pK7FWG2.0 (empty control) and pK7FWG2.0-AtGDSL1-eGFP were transformed into Agrobacterium tumefaciens (GV3101).

Agrobacterium infiltration into three- to four-week-old N. benthamiana leaves was performed as described previously (Wood et al., 2009). Inoculated plants were incubated at 26°C in a growth chamber for 5 days. Then, leaf discs or protoplasts were generated from the leaves, and fluorescence was monitored by confocal microscopy (Leica TCS SPS, Wetzlar, Germany). The fluorescence emissions were at 510–540 nm for eGFP and 658–665 nm for chloroplast, and excitations were at 450–490 nm for eGFP and 630–640 nm for chloroplast.

Cloning of AtGDSL1 and BnGDSL1 and genetic transformations of rapeseed

Cloning, expression vector constructs and genetic transformations of AtGDSL1 and BnGDSL1 genes in rapeseed were performed as described in our previous study (Ding et al., 2019). PCR and RT-PCR analyses, as well as lipase activity assays, confirmed the stable transformation of GDSL1 in rapeseed.

Endogenous SA, JA and PA measurements

SA and JA extraction and quantification were carried out as stated in Li et al. (2011). Approximately 0.5 g of infected leaves collected at 12 and 24 h after inoculation was extracted with 0.5 mL of 1-propanol/H2O/concentrated HCl (2/1/0.002, v/v/v). Then, 25 μL of standard mixture solution from 25 ng of each kind of hormone was added for selection using diagnostic precursor-to-product ion transitions. High-performance liquid chromatography–electrospray ionization–tandem mass spectrometry was performed to separate and quantify the plant hormones obtained from leaf extracts.

PA levels in the leaves of WT and transgenic rapeseed following S. sclerotiorum infection were determined using a plant sandwich enzyme-linked immunosorbent assay (ELISA) kit (GeneTex, San Antonio, TX) (Cornuault and Knox, 2014; Huang et al., 2008; Lee et al., 2013). Briefly, 50 μL of standard or sample diluents was added to the PA antibodies pre-coated microtiter plate wells. After incubating, biotinylated anti-IgG and streptavidin-horse radish peroxidase were added to form immunocomplex. After adding substrate TMB and stop solution, the colour change was measured at 450 nm using a microplate reader (Synergy HT, BioTek, Winooski, VT). The concentrations of PA were then determined by comparing the optical densities of the samples to the standard curve. These experiments were all performed with three biological replicates.

Histochemical staining, and ROS and antioxidative enzyme assays

The growth of S. sclerotiorum as well as H2O2 in the leaves of transgenic plants and non-transformed controls were examined by trypan blue staining and DAB (0.5 mg/ml) staining, respectively, as previously described (Wang et al., 2009). Images were obtained using DM IRR (LEICA, Wetzlar, Germany) and IX73 (Olympus, Tokyo, Japan) invert microscopes under bright-field conditions. Cellular ROS concentrations and SOD and POD activities in the leaves of WT and transgenic rapeseed following S. sclerotiorum infection were determined by sandwich ELISA, as described above. All assays were performed in triplicate with three independent experimental replications.

Lipase activity assay

Lipase activity was estimated colorimetrically by measuring the liberation of p-nitrophenol from p-nitrophenyl derivatives at 405 nm (Ruiz et al., 2004). Briefly, bacterial protein extracts were incubated in the reaction mixture [1 mM p-nitrophenyl derivatives, 5 % (v/v) 2-propanol, 0.6 % (v/v) Triton X-100 and 50 mM Na-phosphate buffer, pH 7.0] at 37°C. Lipase activity was expressed as p-nitrophenol micromol min⁻¹mg⁻¹ protein. Absorbance was measured at 405 nm every 1 h for 7 h. Purified protein concentrations were determined using a micro BCA protein assay kit (Thermo Scientific, Waltham, MA) with bovine serum albumin as the standard. Lipase activity in plants was examined using a lipase activity assay kit (Y-J Biotech, Shanghai, China) based on sandwich ELISA and colorimetry methods. All assays were performed in triplicate with three independent experimental replications.

Association analysis for SSR-resistance traits in B. napus

We selected 324 rapeseed accessions from all over the world to form a natural population. The genotypic data were obtained by 7 × re-sequencing and referring to the genome of ‘Darmor-bzh’. SNPs and indels were tested using the Broad Institute’s open-source Genome Analysis Toolkit (https://software.broadinstitute.org/gatk/). The sites with SNP deletions of more than 0.6 were removed, and then, those sites with minor allele frequency < 0.05 and heterozygosity< 0.25 were removed. The phenotypic data were collected by investigating the disease rate and disease index of mature rapeseeds that were grown at the Yanglou test base (Wuhan, China) from 2015 to 2018. The field experiment was carried out using a randomized block design with
three replicates and three lines per plot. The best linear unbiased prediction of phenotypic data was performed. Combined with SNP genotypic data, a candidate gene association analysis was carried out using the Tassel 5.0 software according to Wang et al. (2017). The threshold was set to $P < -\log_{10}(0.05/SN)$, where N represents the number of valid SNPs.

### Identification of selective sweeps

In this population, rapeseed accessions with disease index and disease rate values in top and bottom 20% were selected and divided into two subgroups, respectively. To identify selective sweeps on Chr.07, a sliding-window approach with 2-kb steps in 20-kb windows was used to calculate $\pi$ ratios ($\pi_1, \pi_2, \pi_3$) and $F_{ST}$ values according to an empirical procedure described by Li et al. (2013a). Sliding windows with significantly low and high $\pi$ ratios (the 5% left and right tails, respectively) and the highest 5% of $F_{ST}$ values of the empirical distribution were considered as selective regions in the genome, which should harbour genes that underwent selection during rapeseed breeding.

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### Author contributions

X.L.T. and S.Y.L. conceived the study. X.L.T. and L.N.D. designed the experiments and wrote the manuscript. X.J.G. M.L., R.L. and X.L.T. and S.Y.L. contributed equally to this work.

### Conflicts of interest

The authors declare no conflicts of interest.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 Enzymatic assay of the AtGDSL1 protein.

Figure S2 Transient expression and disease-resistance effects of AtGDSL1 or BnGDSL1 in N. benthamiana.

Figure S3 Overexpression of AtGDSL1 enhanced resistance to S. sclerotiorum in transgenic rapeseed.

Figure S4 Effects of AtGDSL1 overexpression on SOD and POD activities (a) and the expression of ICS1 (b) after S. sclerotiorum infection.

Figure S5 Endogenous contents of OPDA and JA-ILE in ‘NY12’ and AtGDSL1 transgenic rapeseed plants after S. sclerotiorum infection.

Figure S6 Full length cDNA and deduced amino acid sequences of BnGLIP1.

Figure S7 Alignment of BnGLIP1 with homologs from other plant species.

Figure S8 Response of BnGDSL1 to pathogen infection and phytohormone treatments.

Table S1 Primers used for PCR and qRT-PCR.

Data S1 Genomic regions with strong selective sweep signals on chromosome C07.

Data S2 Allelic variation analysis of BnGLIP1 based on the SNP, GDSL_chrC07_37987173.