

**A critical role for AtGDSL1 lipase gene  
in *Sclerotinia sclerotiorum* resistance  
and functional identification of its  
rapeseed homologue that underwent  
selection during breeding**



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## 01 Background

- ✓ *Sclerotinia* stem rot (SSR), caused by *S. sclerotiorum*, is a devastating disease of oil crops and has a worldwide distribution
- ✓ Studies have demonstrated that *S. sclerotiorum* is a hemi-biotrophic pathogen fungus
- ✓ Progress is slow due to the complex inheritance as well as the lack of resistant germplasm

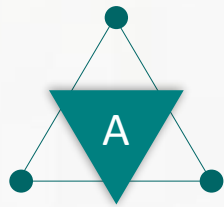




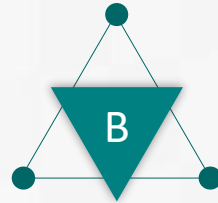
May,15,2019, Nanjing, South of China



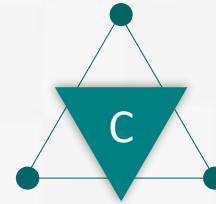
# GDSL-type lipases/esterases



**Pathogen  
defense**



**Seed  
development**



**lipid  
metabolism**

*AtGLIP1/2*  
*CaGL1*  
*CaGLIP1*  
*OsGLIP1/2*

*AtEXL4*  
*AtCDEF1*  
*BnLIP2*

*AtSFAR*、  
*BnSDP1*



**1**

To find out the role for *AtGDSL1*, a novel GDSL-motif lipase, in defense against *S. sclerotiorum* in rapeseed

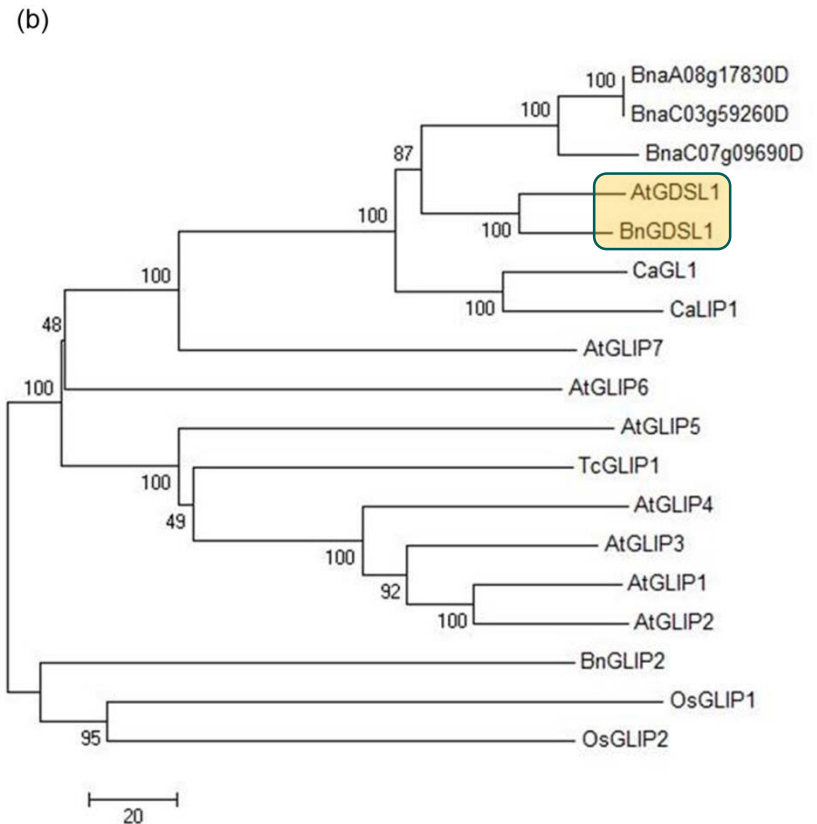
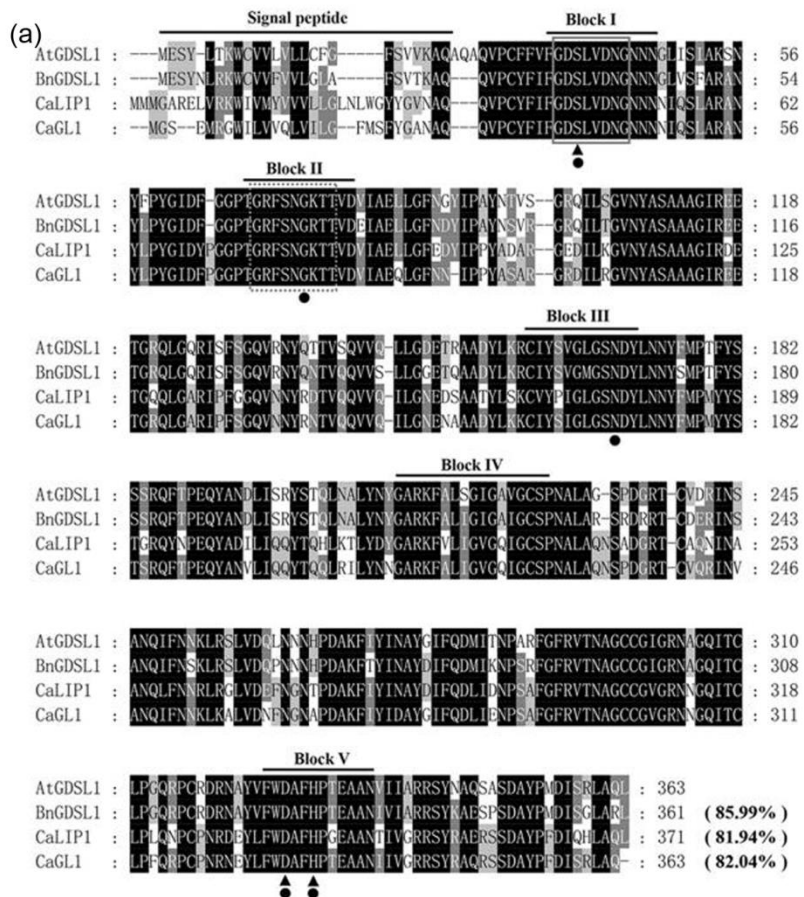
**2**

Practical value to SSR resistance molecular breeding for rapeseeds



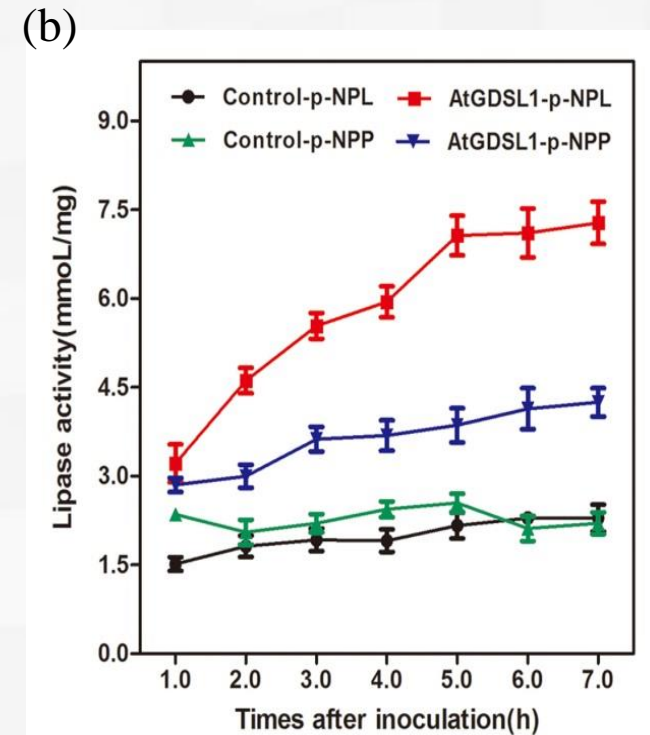
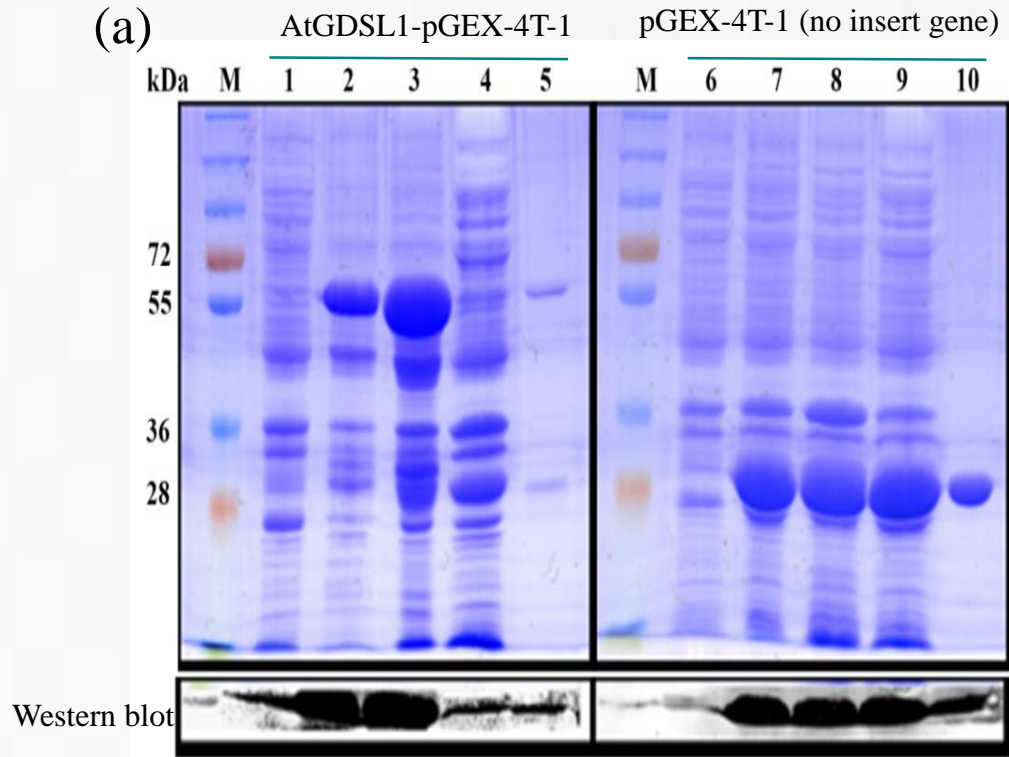
## 3.1

## Sequence comparisons of AtGDSL1 with other GDSL lipases



## 3.2

# AtGDSL1 encodes a GDSL lipase protein

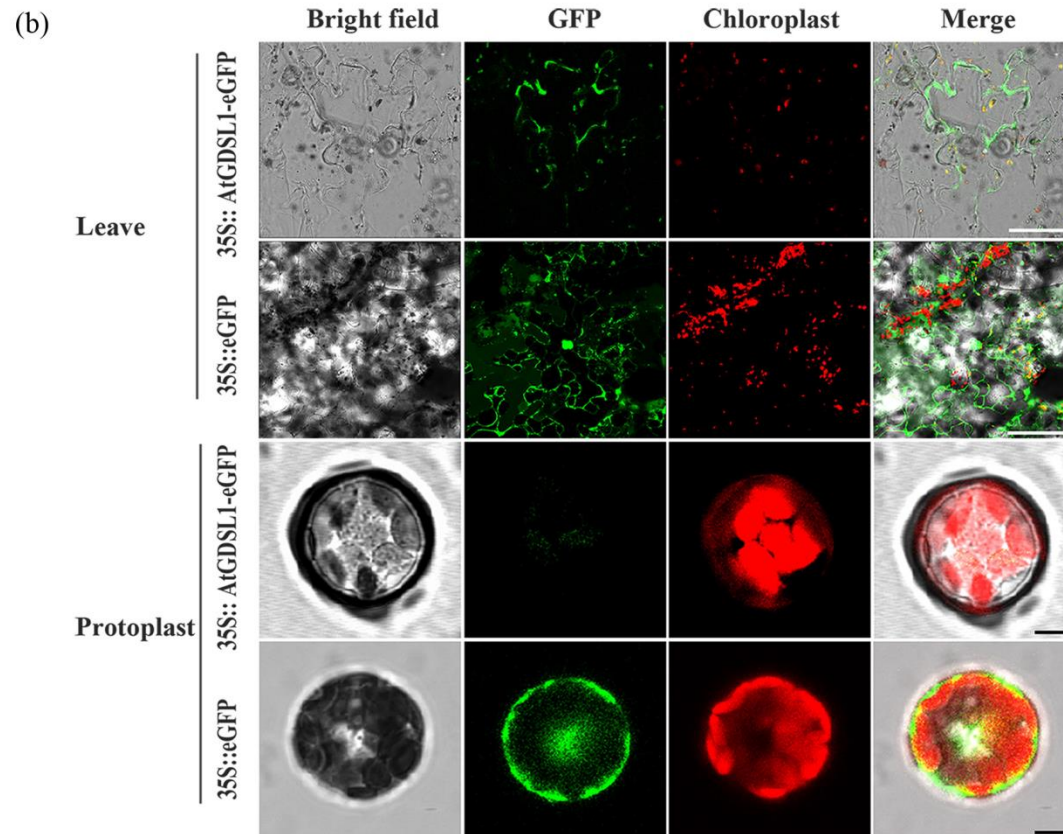
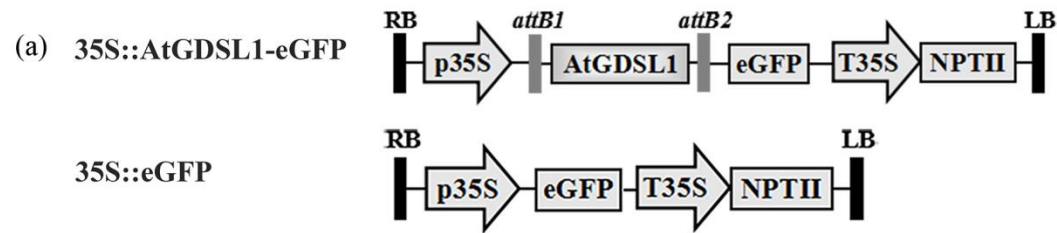


(a) SDS-PAGE of AtGDSL1 protein expressed in *E. coli*

(b) Lipase activity assay of AtGDSL1 protein



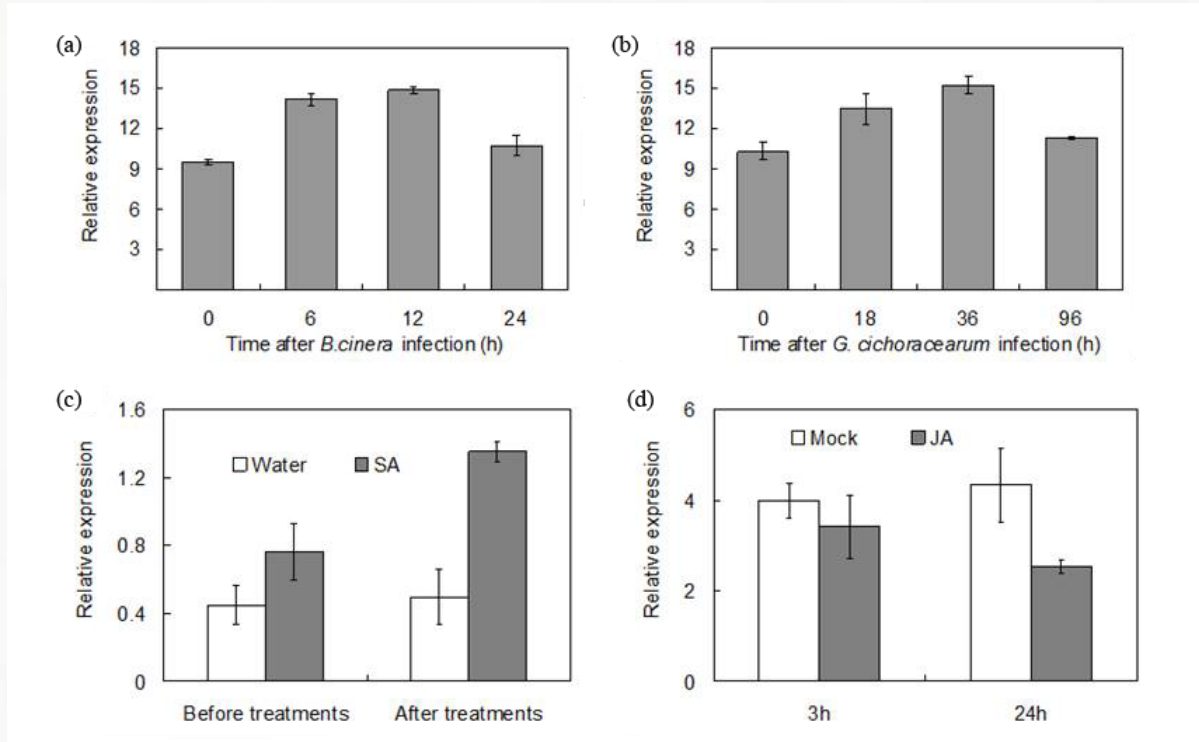
## 3.3

Subcellular localization of *AtGDSL1* in *N. benthamiana* leaves



## 3.4

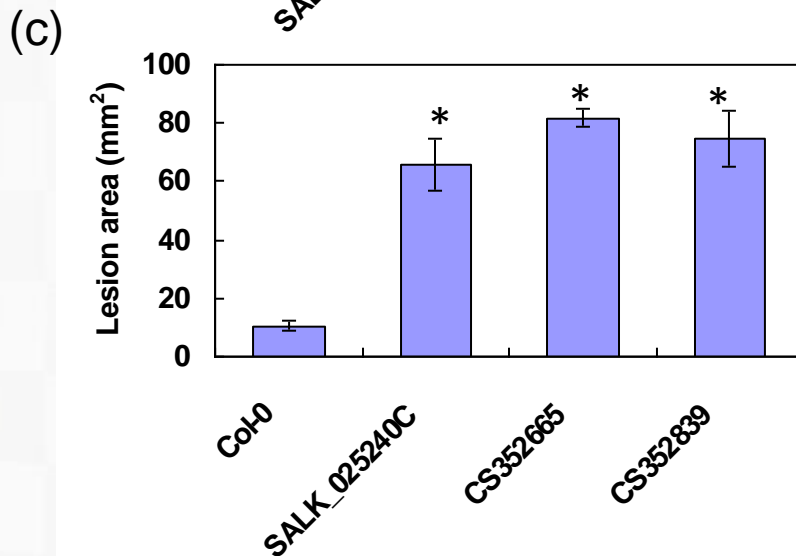
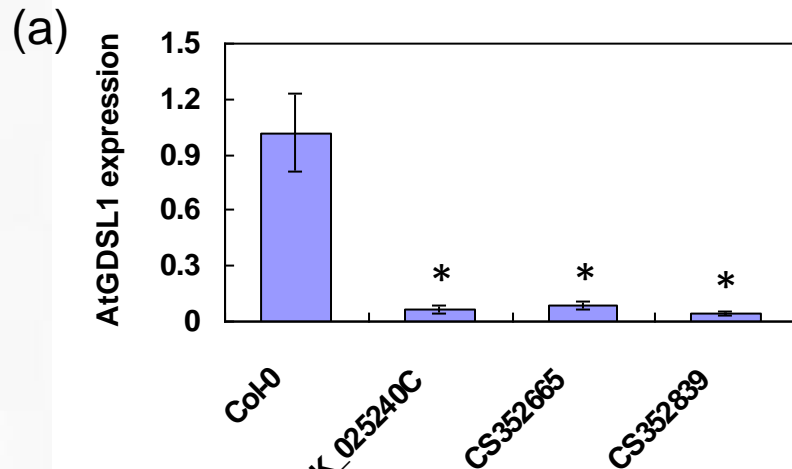
## The insertional mutations of *AtGDSL1* in *Arabidopsis* enhanced susceptibility to *S. sclerotiorum*



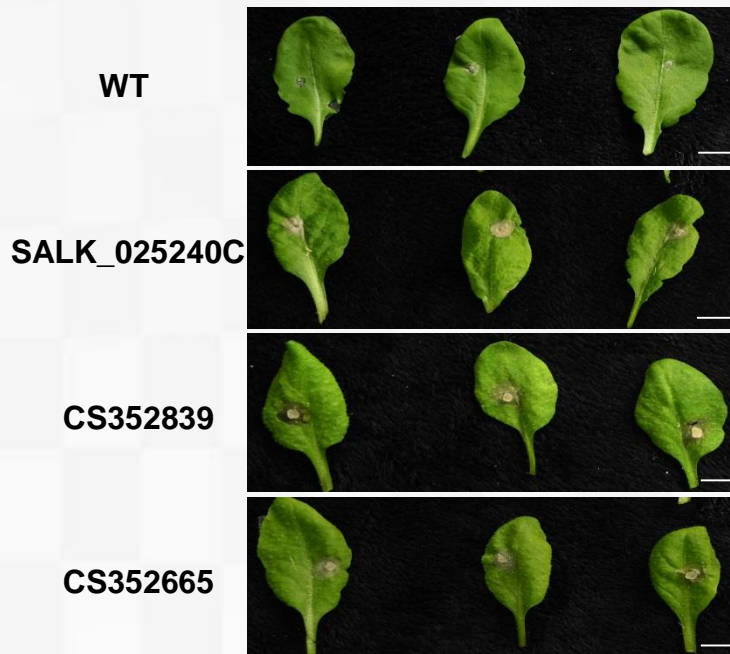
Expression analysis of *AtGDSL1* responding to biotic stresses and defense-related phytohormones based on reported microarray data in *Arabidopsis*



## 3.4

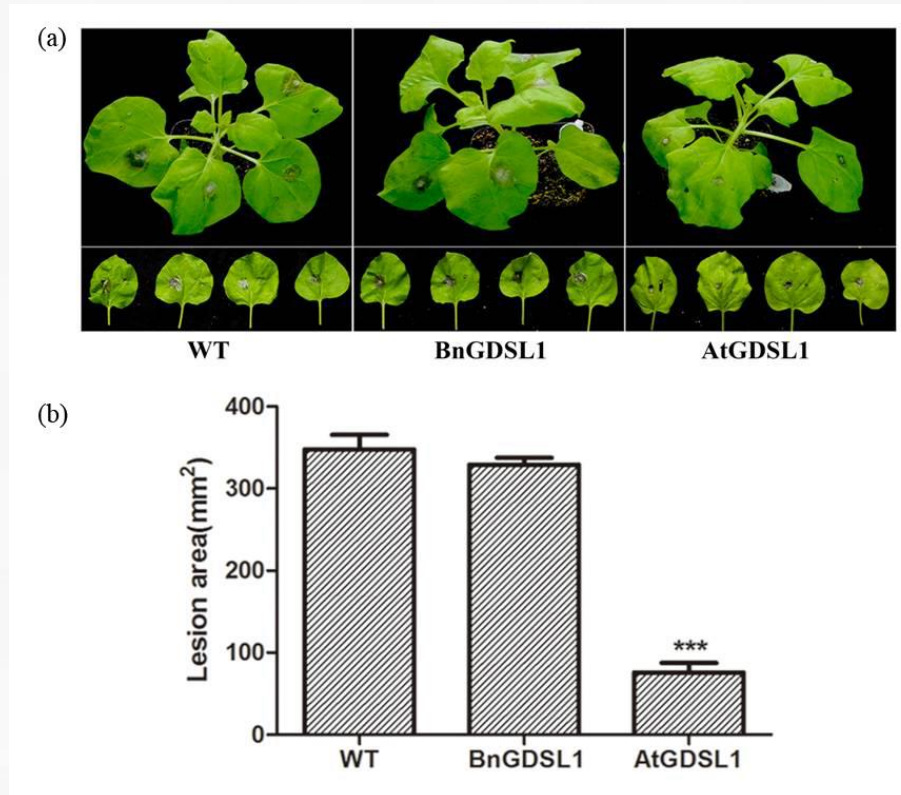
The insertional mutations of *AtGDSL1* in *Arabidopsis* enhanced susceptibility to *S. sclerotiorum*

(b)



## 3.5

## Overexpression of *AtGDSL1* in *B. napus* enhanced resistance to *S. sclerotiorum*

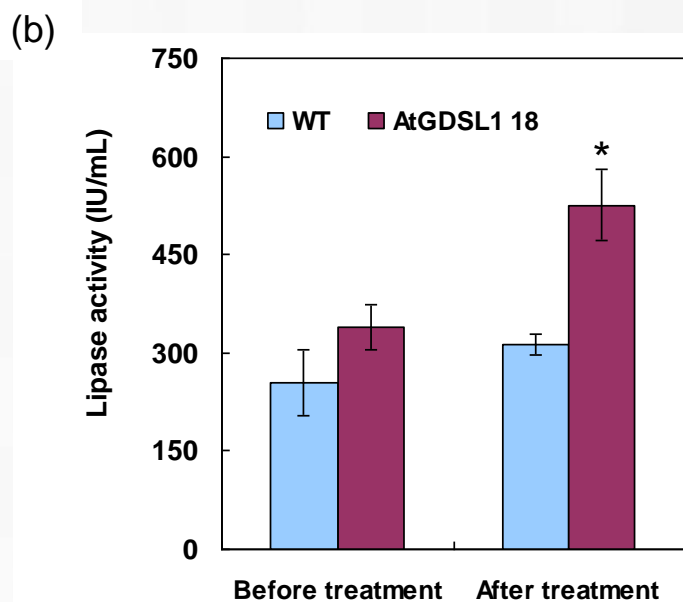
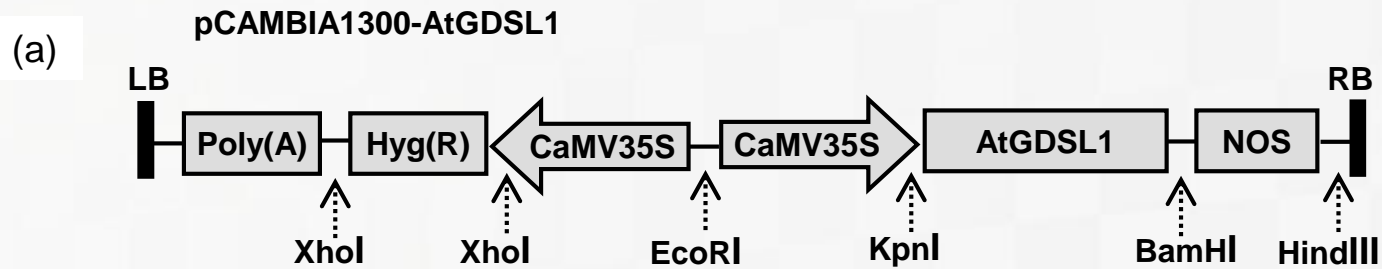


Transient expression and disease-resistant effects of *BnGDSL1* or *AtGDSL1* in *N. benthamiana*



## 3.5

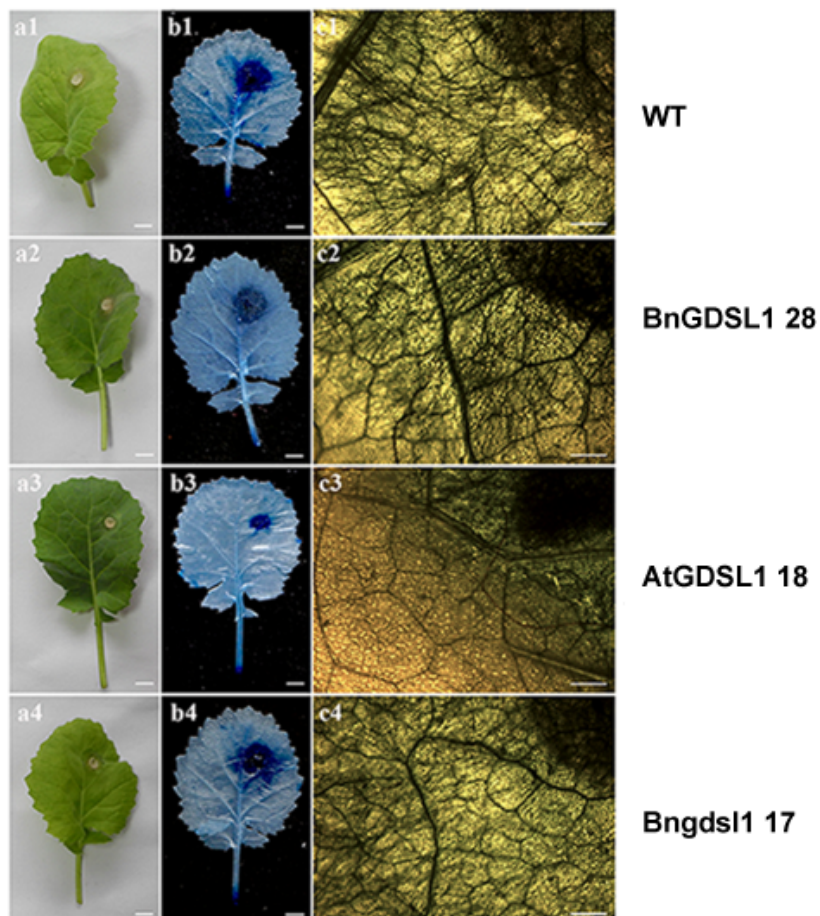
# Overexpression of *AtGDSL1* in *B. napus* enhanced resistance to *S. sclerotiorum*



# 3.5

## Overexpression of *AtGDSL1* in *B. napus* enhanced resistance to *S. sclerotiorum*

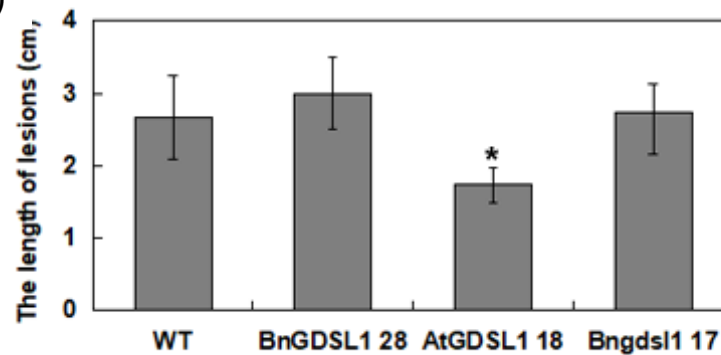
(e)



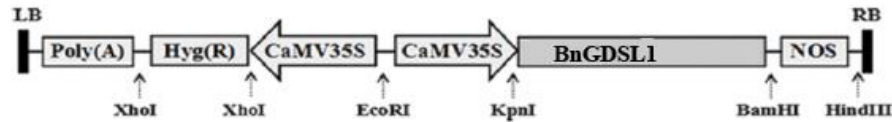
(f)



(g)



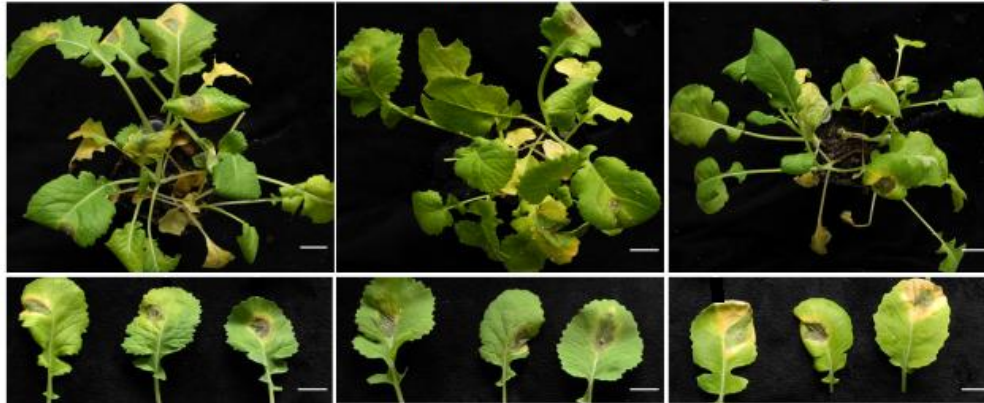
(a) pCAMBIA1300 - BnGDSL1



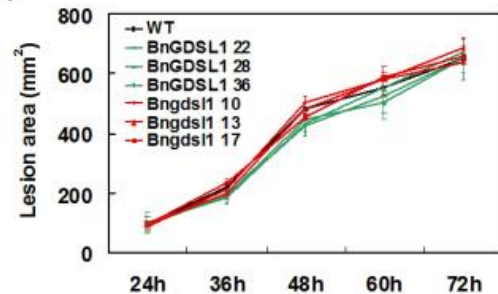
(b) pHellgate 12 - Bngdsl1



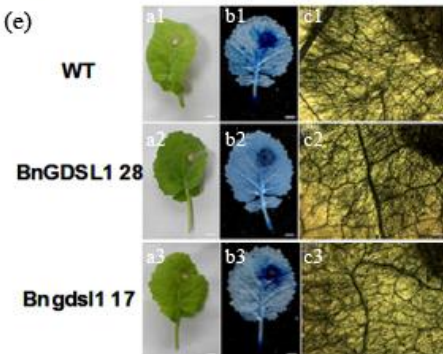
(c) WT BnGDSL1 28 Bngdsl1 17



(d)



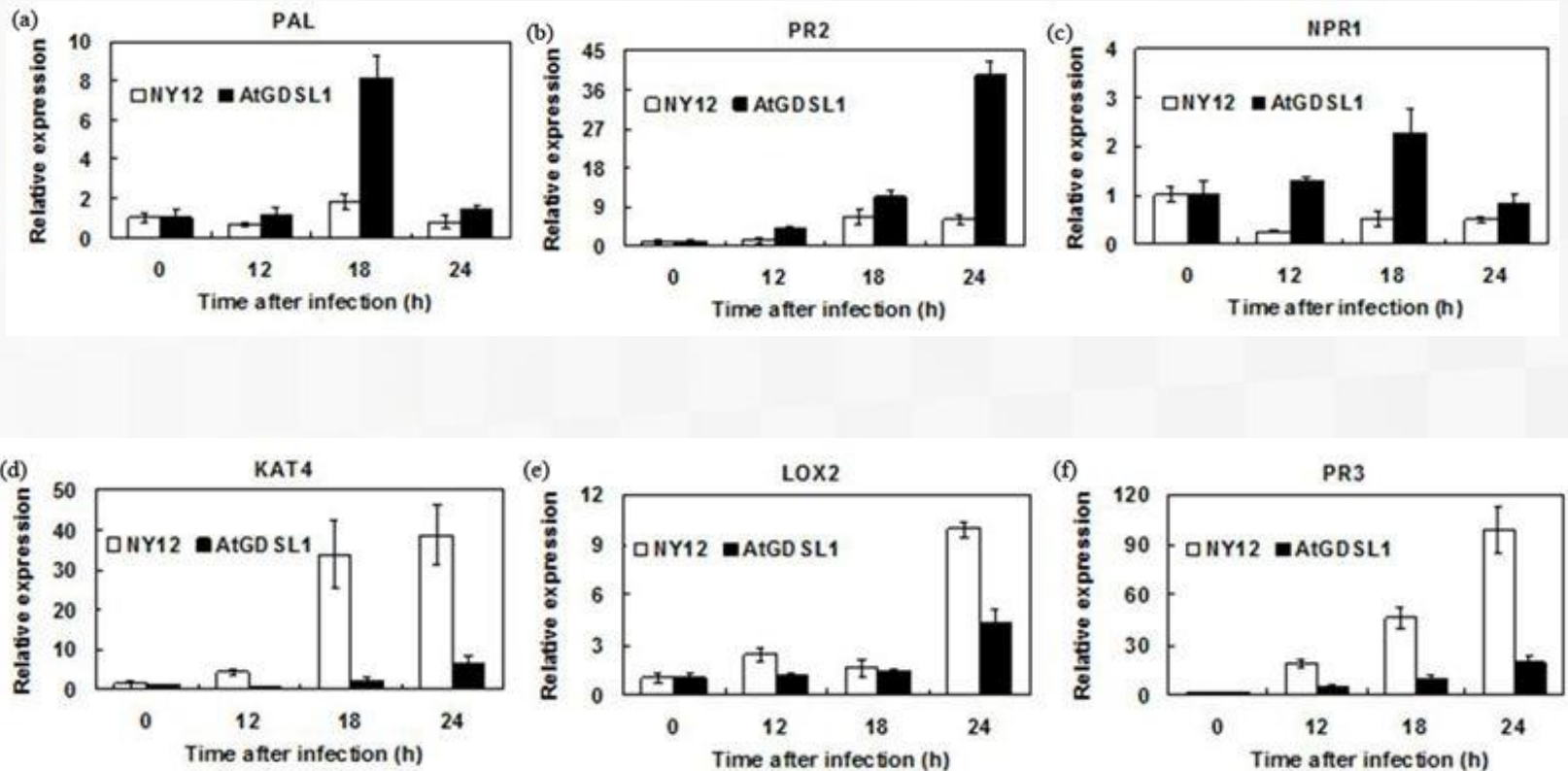
(e)



Transformation of rapeseed with *BnGDSL1* and the disease symptoms of transgenic plants inoculated with *S. sclerotiorum*

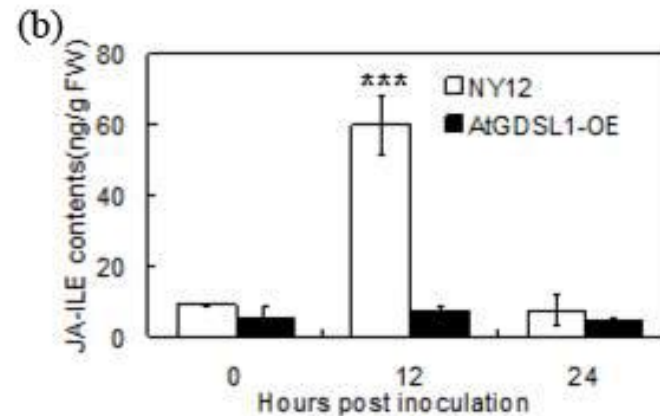
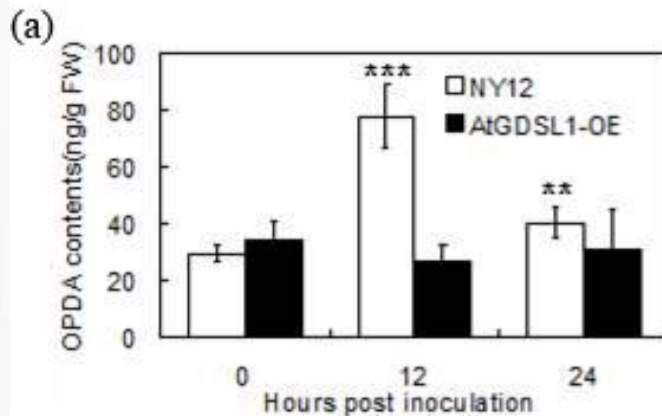
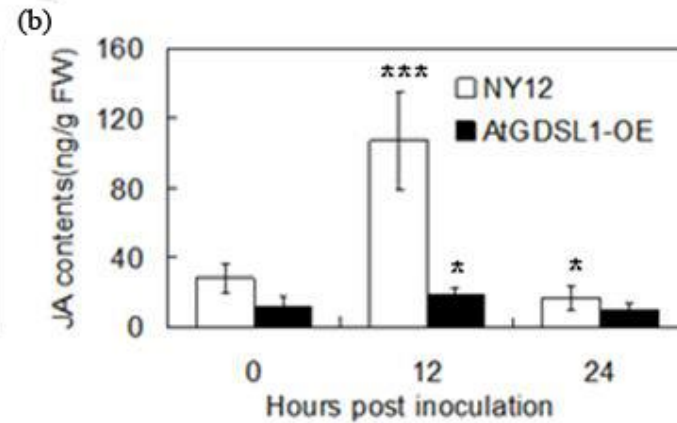
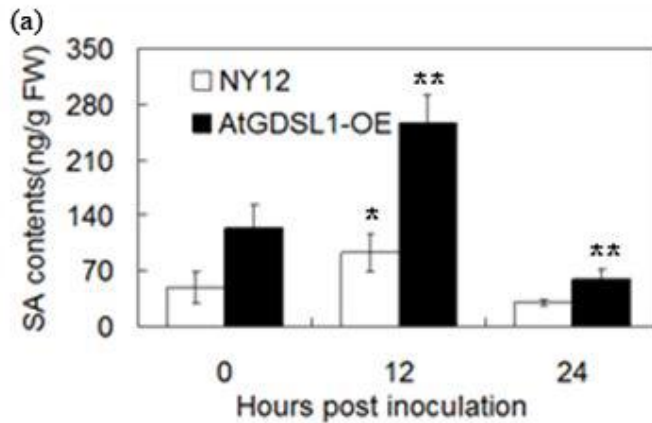


## 3.6

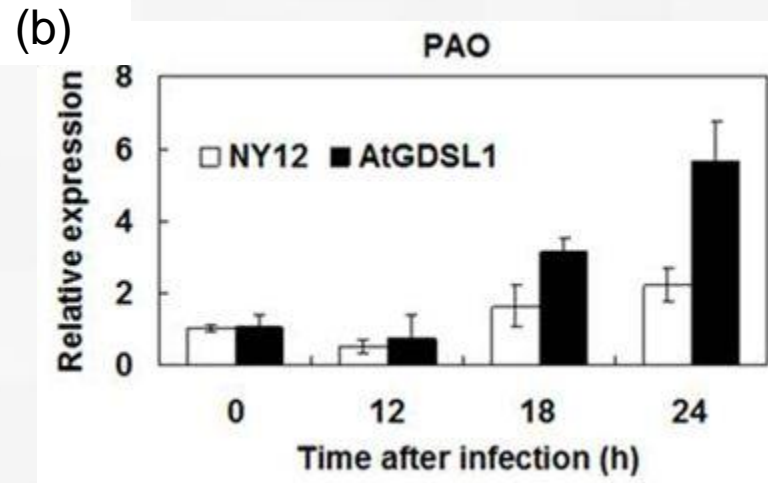
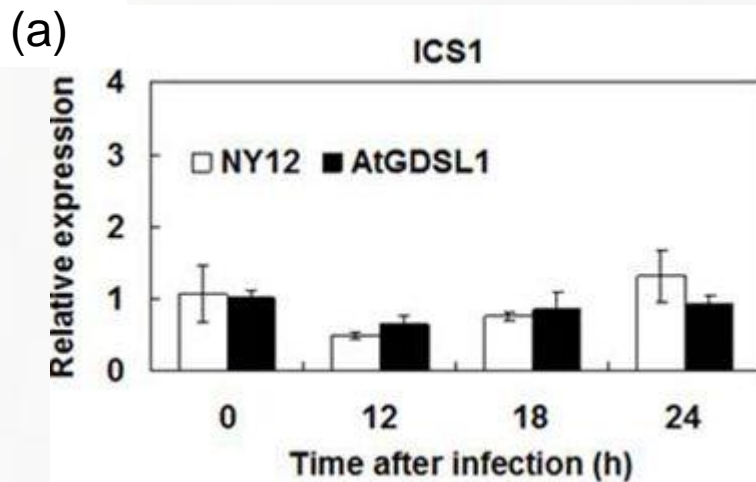
*AtGDSL1* regulates the expression of SA- and JA-related genes

## 3.7

## SA and JA contents changed differently in WT and transgenic plants after the infection



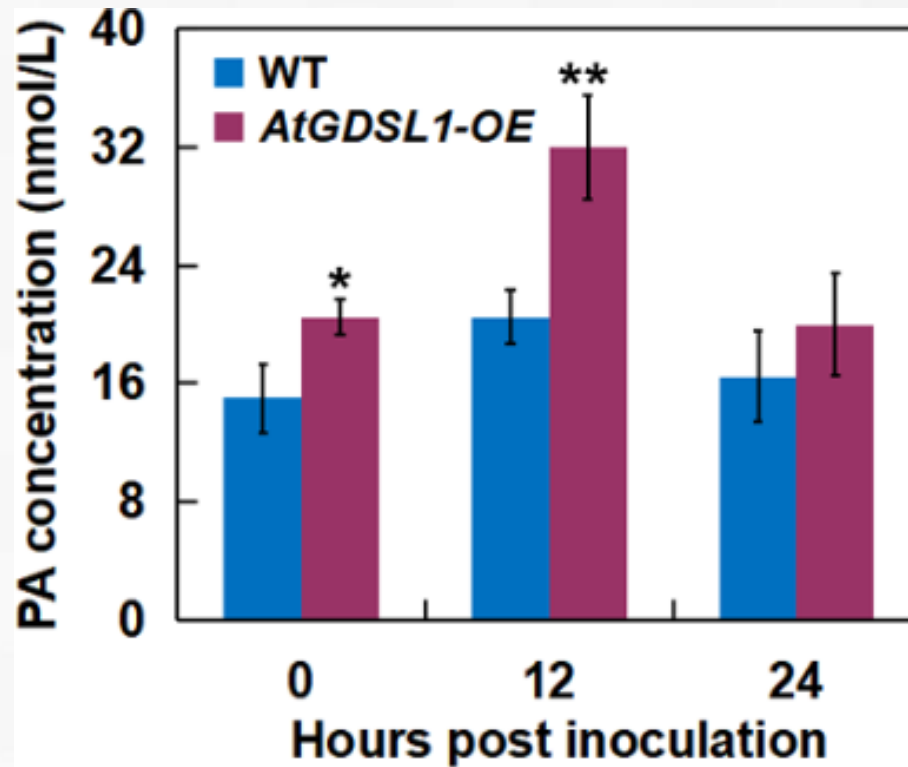




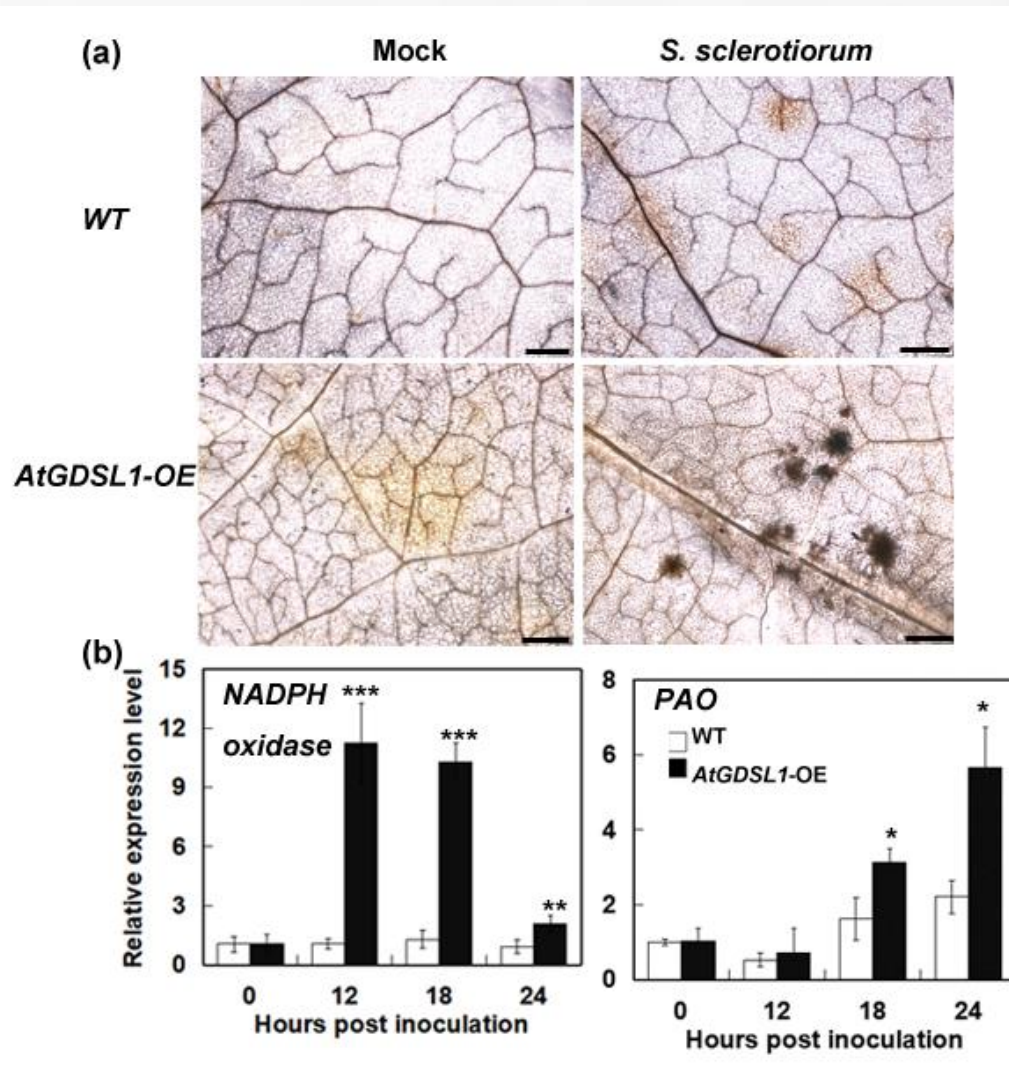
- ◆ AtGDSL1 can directly or indirectly affect SA biosynthesis mainly through the PAL pathway
- ◆ The expression pattern of *PAO* is similar to those of SA-response genes

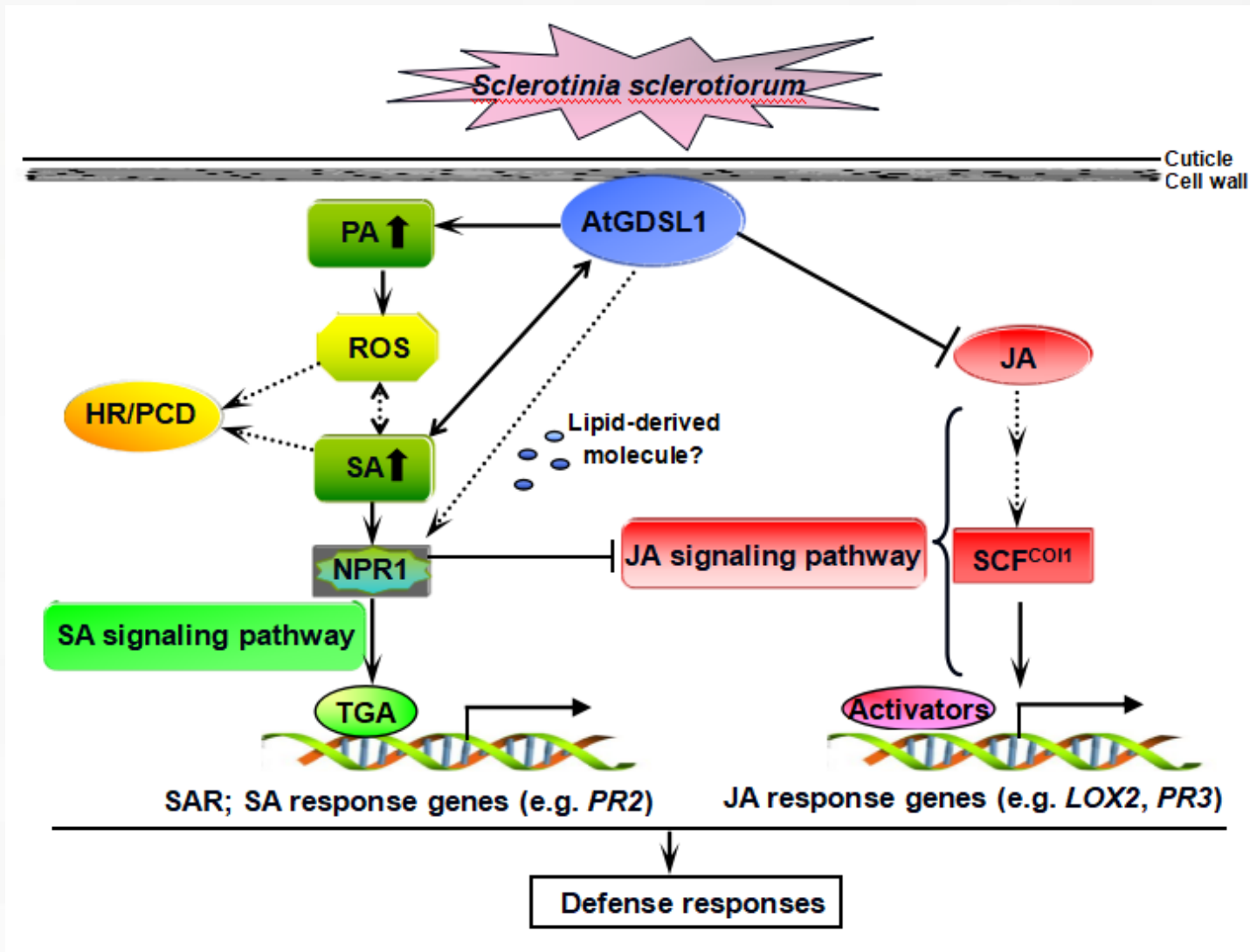


## Overexpression of AtGDSL1 enhances the PA level



# Overexpression of AtGDSL1 promotes ROS accumulation.





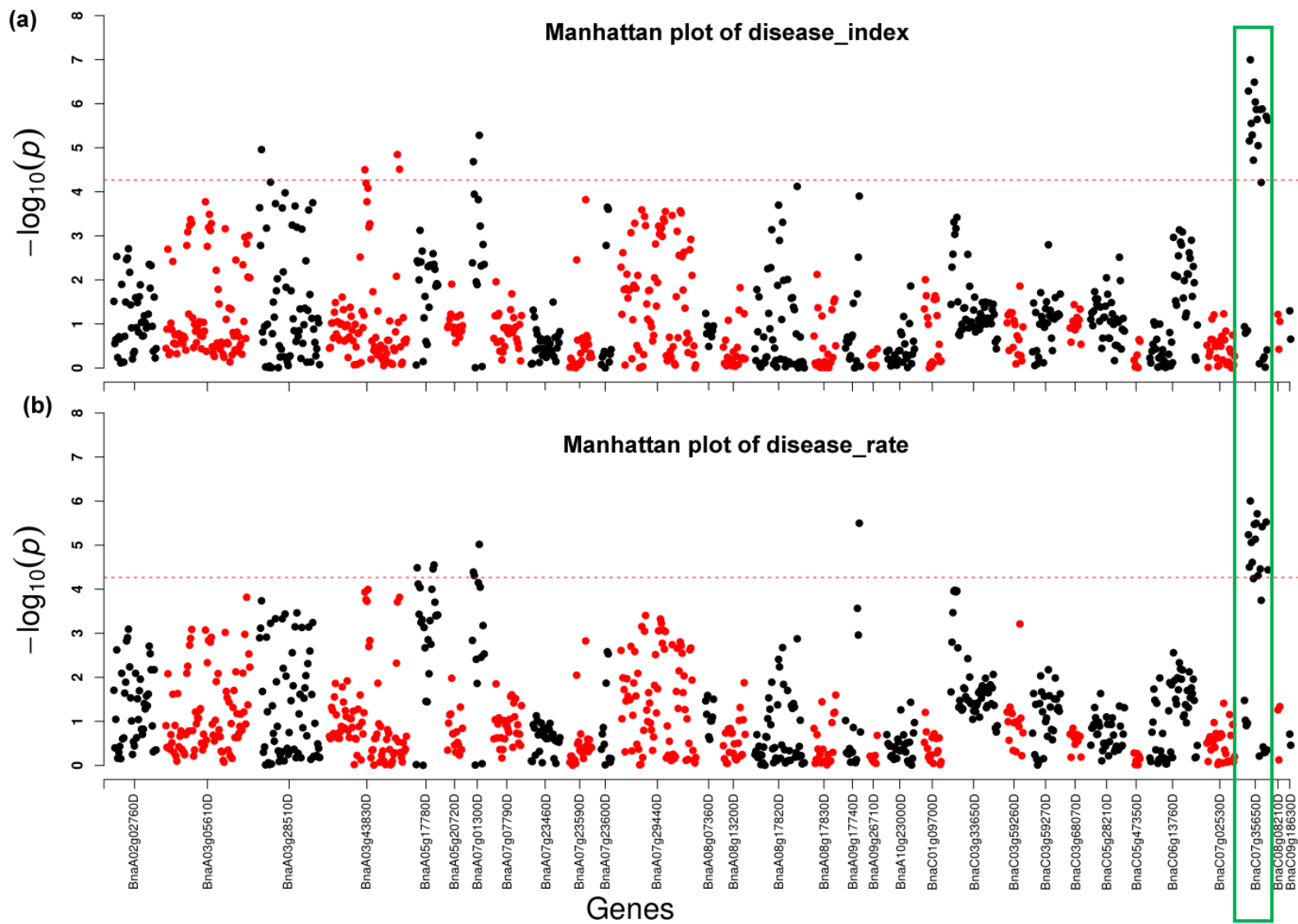
Proposed model for AtGDSL1 action in SSR resistance



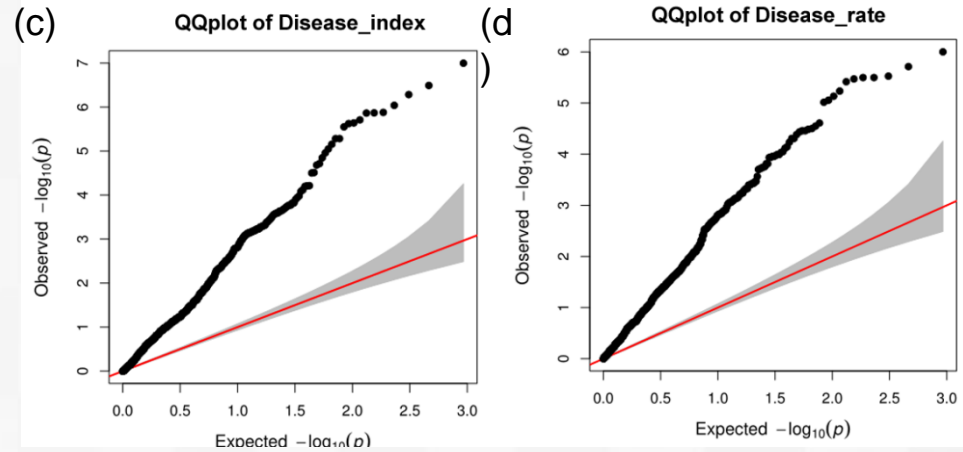
# Seeking the counterpart of AtGDSL1 in *Brassica napus* genome



## 4.1

Candidate gene association analysis of AtGDSL1 homologous genes in a natural *B. napus* population

	Disease rate	Disease index
Genes	<i>BnGLIP1</i>	<i>BnGLIP1</i>
SNP	14	15
Contribution rate	6.8-9.13%	7.40-10.49%



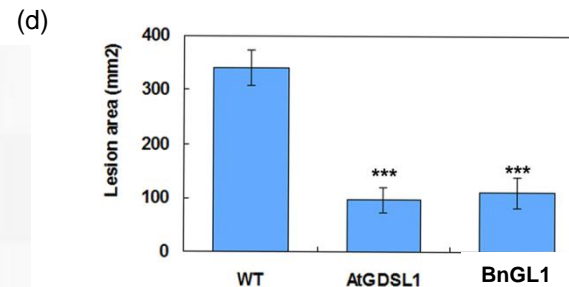
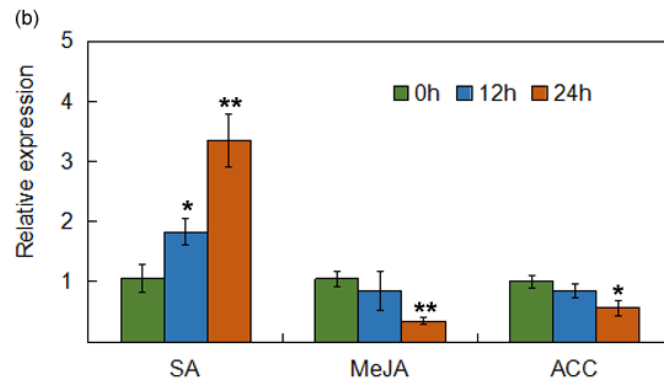
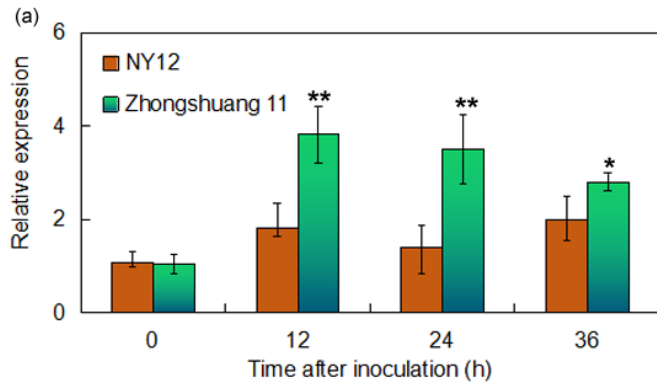
(a) and (b) The abscissa represents genes, separated by black and red color intervals. Each point represents a SNP, and the SNP that exceeds the threshold red line  $-\log_{10}(P)$  ( $P=0.05$ ) is significant.

(c) and (d) The Q-Q plots for incidence and disease index of SSR from association analysis. The red line was the unbiased estimates of the expected and observed value.



## 4.2

# *BnGLIP1* may be the main functional gene among the homologues in SSR-resistance

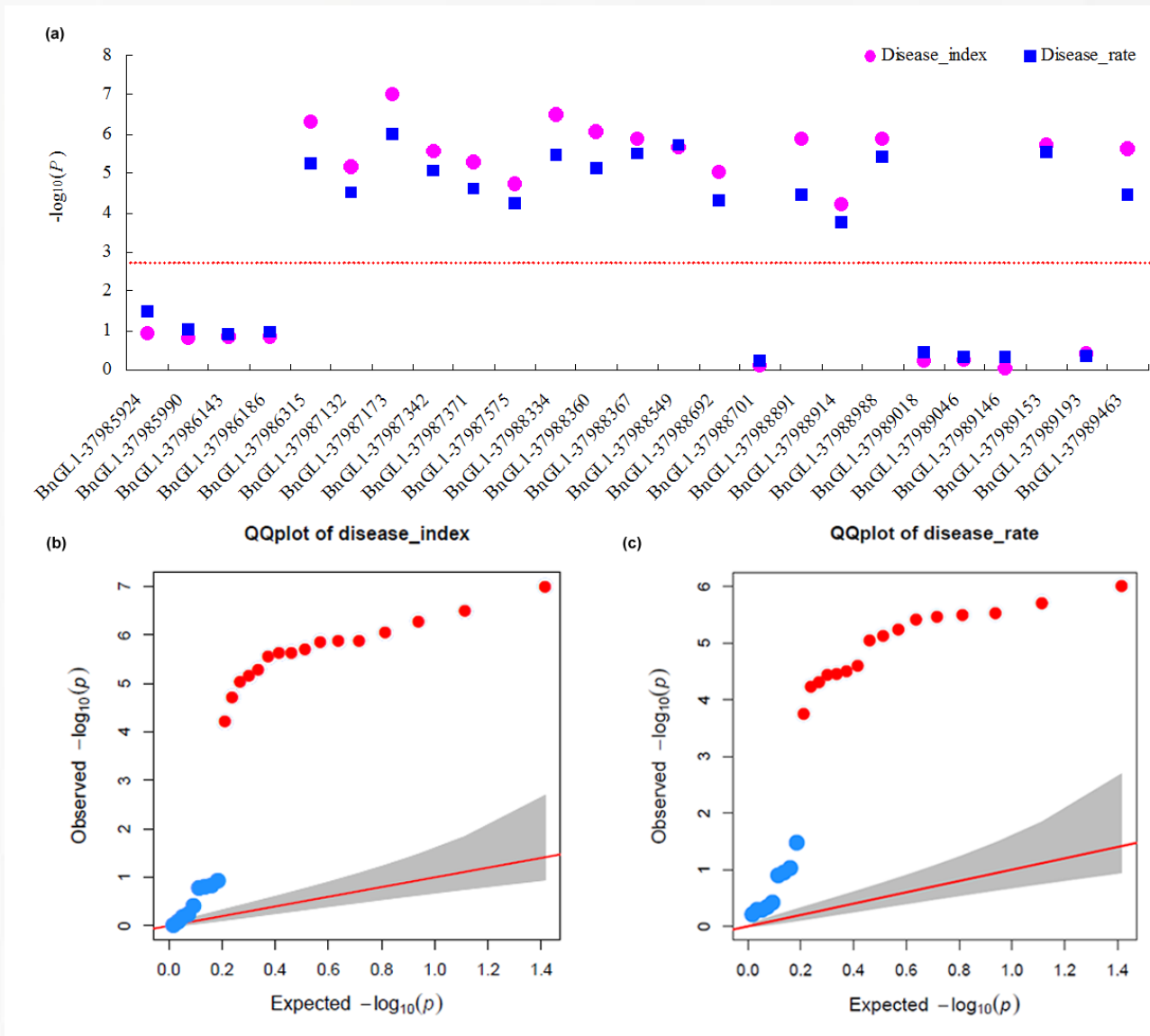


(a) and (b) *BnGLIP1* responses to *S. sclerotiorum* infection and plant hormone treatments in *B. napus*

(c) and (d) Transient expression and disease-resistant effects of *BnGLIP1* or *AtGDSL1* in *N. benthamiana*





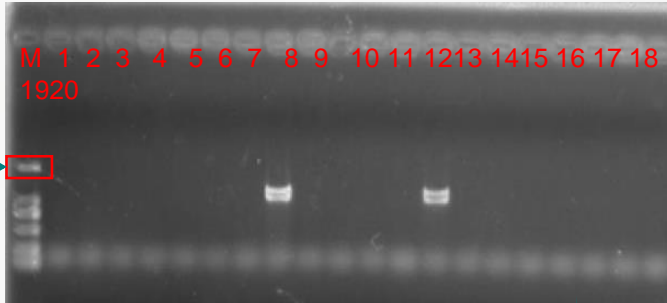


**Table 1 Sixteen significantly associated SNPs detected in BnaC07g35650D**

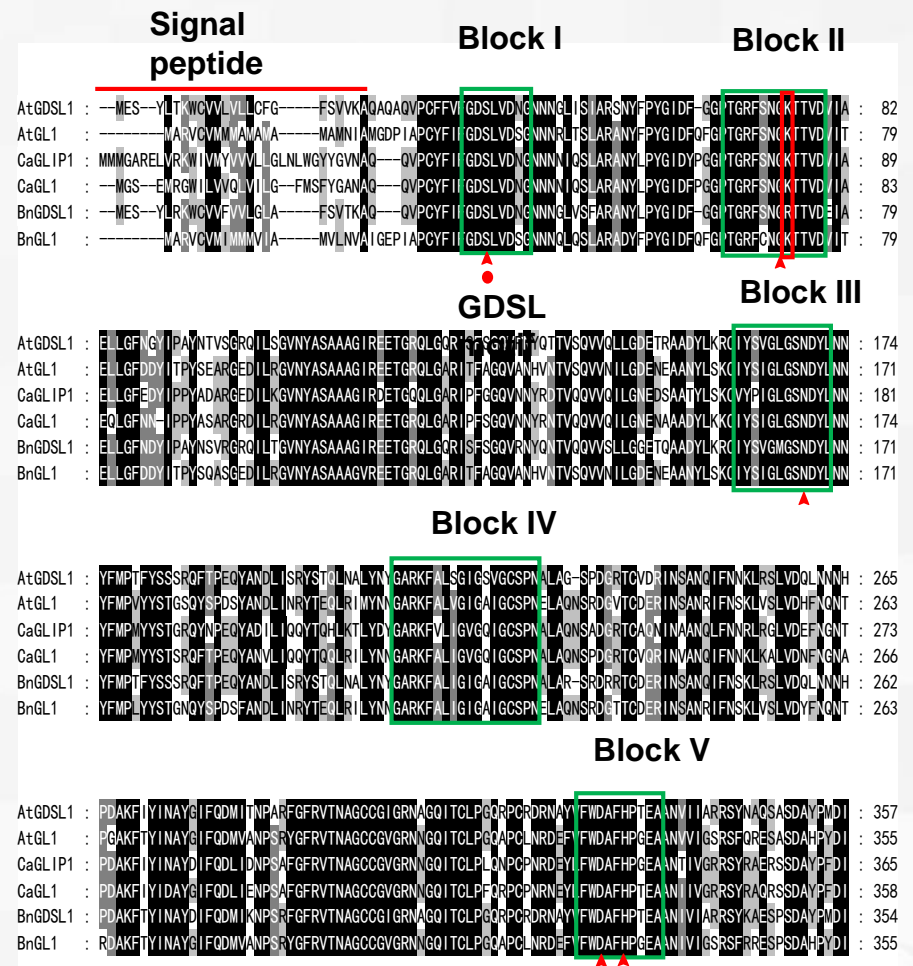
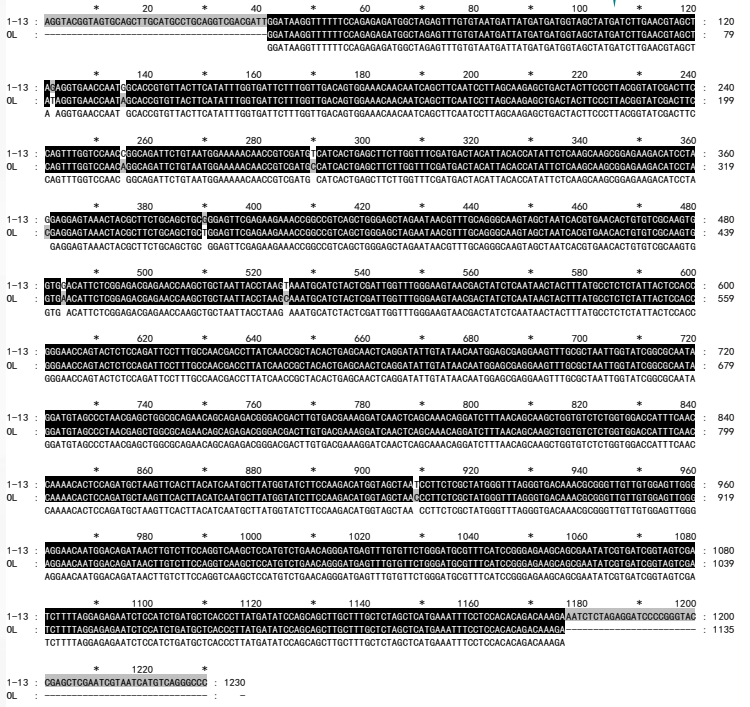
SNPs	Position	Contribution rate (%)		Genotype			cSNP type
		disease_ index	disease_ rate	R	S	Cloned cDNA	
chrC07_37986315	3'-UTR	9.5	8.1	G	C	–	–
chrC07_37987132	exon	8.0	7.1	T	C	T	s
chrC07_37987173	exon	10.4	9.1	G	A	G	ns
chrC07_37987342	exon	8.5	7.8	G	T	G	s
chrC07_37987371	exon	8.2	7.2	G	C	G	ns
chrC07_37987575	exon	7.4	6.7	C	A	C	s
chrC07_37988334	5'-UTR	9.8	8.4	G	T	–	–
chrC07_37988360	5'-UTR	9.2	8.0	A	G	–	–
chrC07_37988367	5'-UTR	9.0	8.4	G	A	–	–
chrC07_37988549	5'-UTR	8.7	8.7	G	T	–	–
chrC07_37988692	5'-UTR	7.9	6.8	A	A/W	–	–
chrC07_37988891	5'-UTR	9.0	7.0	R	T	–	–
chrC07_37988914	5'-UTR	6.5	6.2	M	T	–	–
chrC07_37988988	5'-UTR	9.0	8.3	G	M/A	–	–
chrC07_37989153	5'-UTR	8.8	8.5	M/A	G	–	–
chrC07_37989463	5'-UTR	8.6	7.0	T	C	–	–



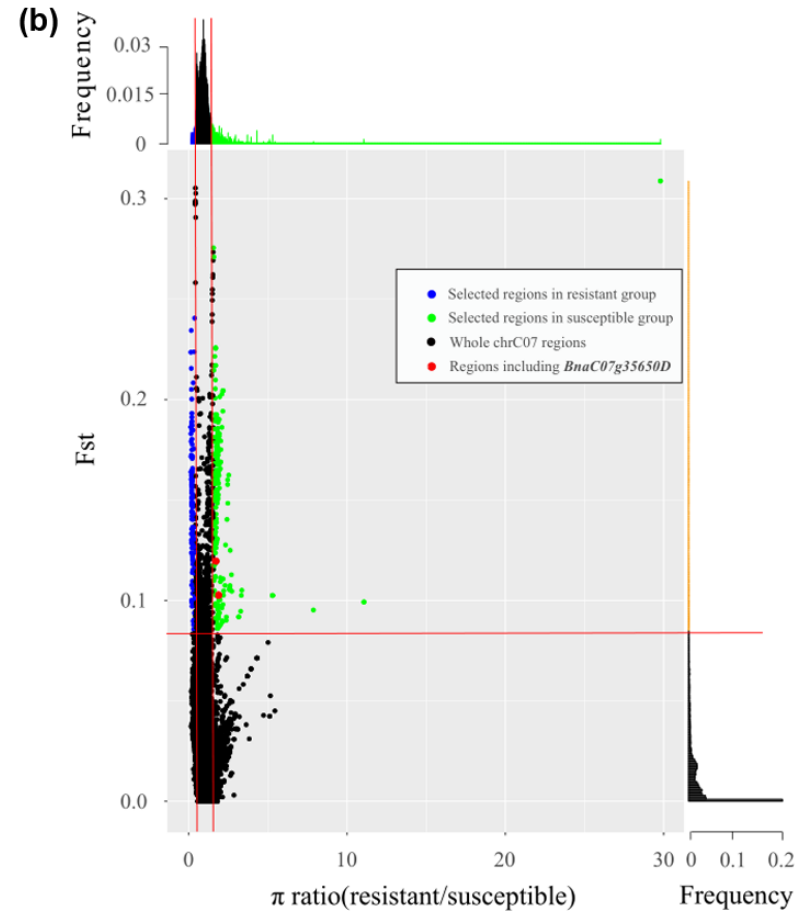
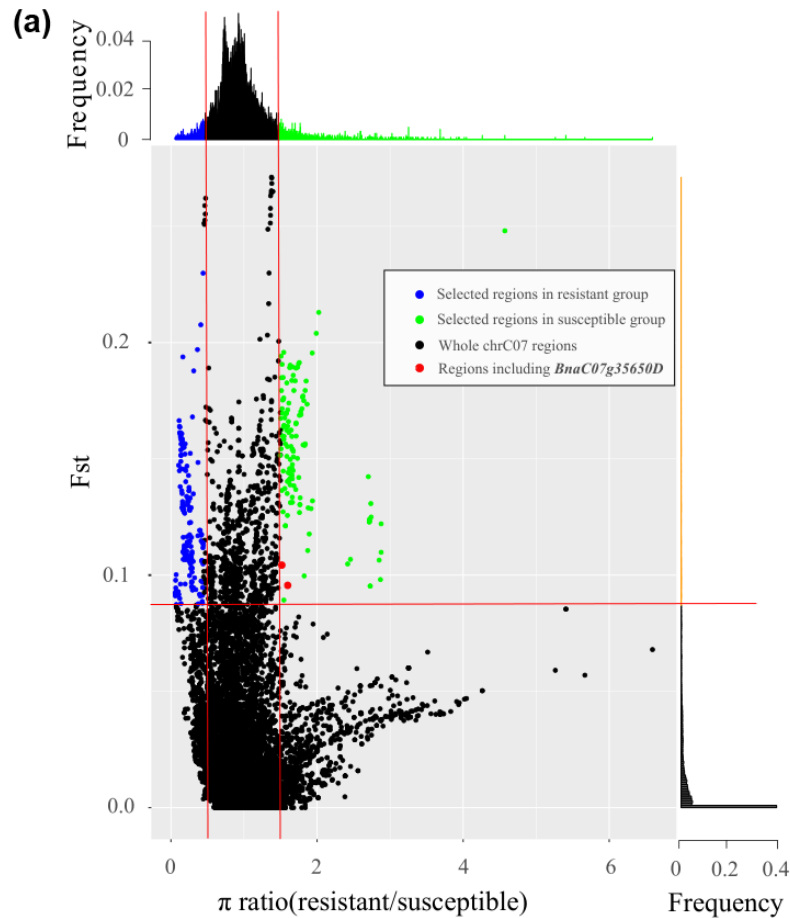
# Functional SNP discovery in candidate gene BnGLIP1



2000bp

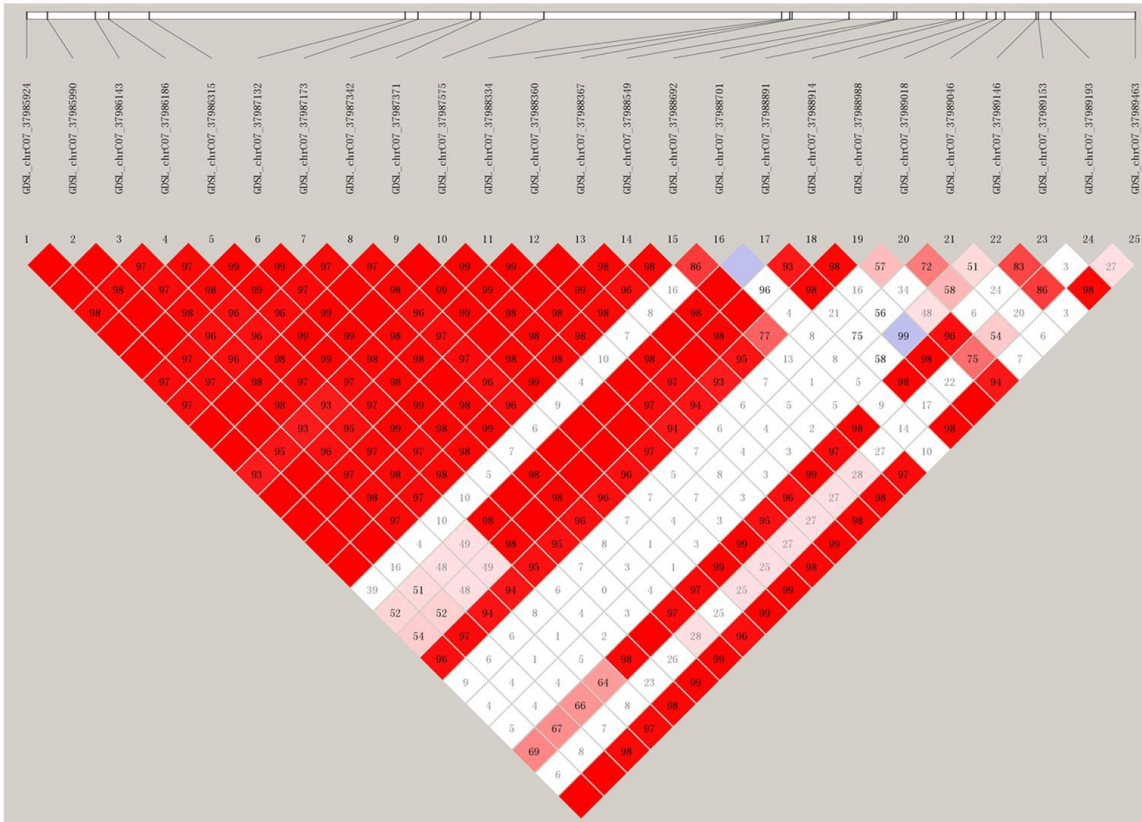


# *BnGLIP1* gene was harbored in the selective sweeps during rapeseed breeding

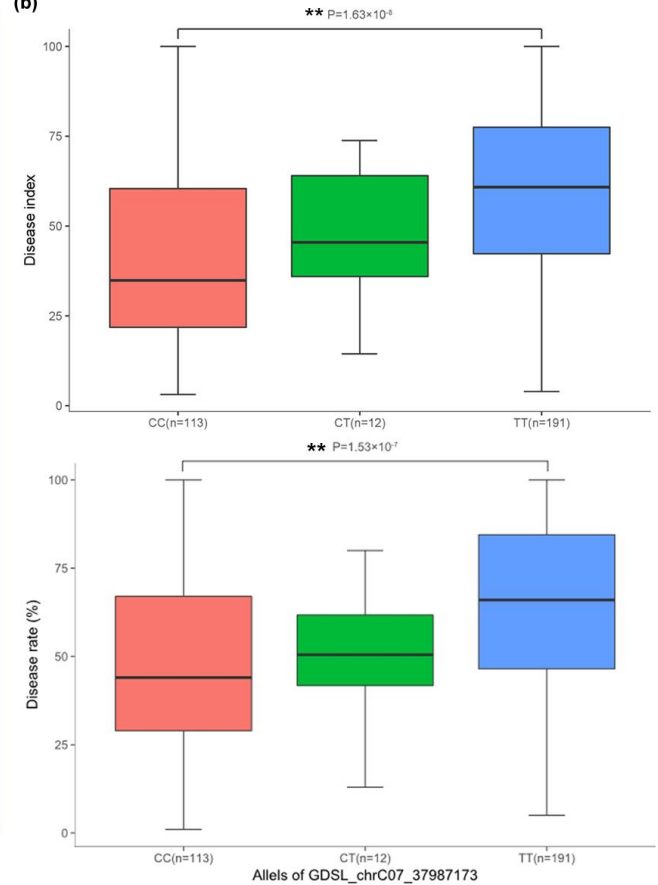


# Allelic variation analysis for *BnGLIP1*

(a)



(b)



*BnGLIP1* locus has great application spatial in improving *S. sclerotiorum*-resistance.



- **At GDSL is an extracellular protein.**
- ***AtGDSL1* confers resistance to *S. sclerotiorum* in *B. napus* by enhance the SA signal pathway and suppressing the JA pathway while it more similar *BnGDSL1* has not the functions.**
- **Candidate gene association study shows *BnGLIP1* instead of *BnGDSL1* is the counterpart of *AtGDSL1* in *B.napus* genome has the function of *S. sclerotiorum* resistance.**
- ***BnGLIP1* locus was selected during the domestication and has great application potential in improving *S. sclerotiorum*-resistance breeding.**



# Acknowledgements



**Li-Na Ding, Xiao-Juan Guo  
Ming Li**



**Sheng-Yi Liu**



**Liang Guo**

**NSFC**

**National Key R & D  
Program of China**





**Thank you for  
your attention**

