# The *Sm* Gene Conferring Resistance to Gray Leaf Spot Disease Encodes a NBS-LRR Plant Resistance Protein in Tomato

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# Abstract

Gray leaf spot (GLS), caused by *Stemphylium lycopersici* (*S. lycopersici*), is one of the most devastating diseases in tomato (*Solanum lycopersicum*). The resistance (R) gene, *Sm*, conferring high resistance to *S. lycopersici,* was introgressed into cultivated tomatoes from the wild tomato species *Solanum pimpinellifolium* (*S. pimpinellifolium)*. Recently, several studies reported the mapping of the *Sm* gene. To date, however, it has not been cloned yet. Here, we cloned this resistance gene using a map-based cloning strategy. The *Sm* gene was mapped in a 160 kb interval of Chromosome 11 between two markers, M390 and M410, by using an $F_2$ population from a cross between the resistant cultivar 'Motelle' (Mt) and susceptible line 'Moneymaker' (Mm). Three clustered NBS-LRR resistance genes, *Solyc11g020080* (*R1*), *Solyc11g020090* (*R2*) and *Solyc11g020100* (*R3*) were identified in this interval. Nonsynonymous SNPs were identified only in the ORF of *R3*, supporting it may be a strong candidate gene for *Sm*. Furthermore, gene silencing of *R3* abolished the high resistance to *S. lycopersici* in Motelle, demonstrating that it is the gene that confers high resistance to *S. lycopersici*. The clone of *Sm* gene will provide new opportunities for innovative breeding strategies to breed multi-resistant tomato cultivars.


# Key Message

Gray leaf spot (GLS) resistance tomato is controlled by one major dominant locus, *Sm.* The *Sm* was fine mapped, and a NBS-LRR gene *Solyc11g020100* was identified as a candidate gene for *Sm*. Further functional analysis supported that it is the gene that confers high resistance to *S. lycopersici* in tomato.

# Introduction

Tomato is one of the most widely cultivated vegetable crops in the world. In recent years, gray leaf spot disease has become prevalent with the continuous increase in tomato cultivation area and has caused severe yield losses in many countries (Everts., 2001; Liu et al., 2004), especially in warm and humid areas. Tomato gray leaf spot disease was considered to be caused by two epidemic species (*S. solani* and *S. lycopersici*) of *Stemphylium* (Miranda et al., 2010; Li et al., 2010; Chai et al., 2015). To date, only one resistance (R) gene, *Sm*, conferring high resistance against gray leaf spot disease, has been identified in the wild tomato species *S. pimpinellifolium* (Andrus et al., 1942; Hendrix et al., 1949). This resistance gene was transferred into the cultivated variety 'Motelle' (comprising the *Sm* gene). The resistance conferred by *Sm* has not been overcome by other epidemic species of *Stemphylium* spp. In a previous study, Behare et al. mapped the *Sm* gene to chromosome 11 with the two flank markers TG110 and T10, and the linkage distances were 4.1 cM and 6.8 cM, respectively (Behare et al., 1991). Several studies have confirmed the *Sm* locus on chromosome 11. However, the *Sm* gene has not been cloned yet, limiting the utilization of this gene in tomato breeding (Yang et al., 2017; Su et al., 2018; Ji et al., 2009 ).

To date, lots of resistance (R) genes have been reported and cloned in plants. The nucleotide binding site-leucine-rich repeat (NBS-LRR) proteins are one of the most important protein family involved in disease resistance in plants. Although it is a very complicated process, it is well known that the defense response

mediated by NBS-LRR proteins can induce effector-triggered immunity (ETI). NBS-LRR proteins share a conserved NBS domain, a C-terminal LRR domain, and a variable N-terminal domain. The NBS module can be further divided into an NB domain (NBD), a helical domain (HD1), and a winged-helix domain (WHD) (Song et al., 2020). Recent structure and functional studies have demonstrated that the NBS-LRR protein ZAR1 can act as a calcium-permeable cation channel to trigger immunity and cell death (Bi et al., 2021). A large number of NBS-LRR proteins have been identified in different disease resistance processes, including *Pi64* conferred resistance to rice blast (Ma et al., 2015), cotton Verticillium wilt resistance (*GbRVd*) (Yang et al., 2016), tobacco resistance to *P. parasitica* (*VaRGA1*) (Ding et al., 2017), wheat powdery mildew resistance (*Pm3b* and *Pm8*) (Hurni et al., 2013), and the Arabidopsis proteins *RPP8* conferred resistance to different viruses (Cooley et al., 2000). In tomato, several NBS-LRR genes have been reported in various defense responses. For example, the CC-NBS-LRR proteins *Ph-3, I-2* and *Mi-1* conferred resistance to *Phytophthora infestans*, *Fusarium oxysporum* and root-knot nematodes, respectively (Sharma et al., 2014).

Breeding new tomato varieties with high resistance to gray leaf spot disease is one of the most important approaches for controlling this disease. Traditional breeding has the disadvantages of being time-consuming, labor-consuming and being limited by a long breeding cycle. Marker-assisted selection (MAS) is an effective tool to screen germplasm and to shorten the process of plant breeding. As mentioned above, however, the *Sm* gene has not been cloned, which limits the utilization of this resistance gene in tomato breeding. The objective of this research was to clone the *Sm* gene. The *Sm* gene was mapped within a 160-kb region of chromosome 11 in the tomato genome. In this interval, a NBS-LRR gene *Solyc11g020100* was identified as a candidate gene for *Sm*. Further functional analysis supported that it is the gene that confers high resistance to *S. lycopersici* in tomato.

# Materials And Methods

### Plant materials

A total of *196* diverse accessions were used in a GWAS for the mapping of the resistance gene *Sm*. The resistant line ‘Motelle’ was crossed with the susceptible line ‘Moneymaker’ to produce $F_1$ and $F_2$ plants for fine mapping and inheritance studies. In addition, $F_3$ plants derived from 13 $F_2$ recombinants were tested for gray leaf spot disease resistance. All the plant materials were grown in a greenhouse with a 16-h light and 8-h dark photoperiod at 25-28 °C.

### GWAS analysis and marker development

The method of GWAS sequencing and data analysis referred to Lin et al. (2014) and Kang et al. (2010). DNA of three- to four-leaf-stage tomato seedlings was extracted according to the CTAB method (Fulton et al., 1995). Molecular markers of SNPs used in this study were based on genome resequencing data with 200-300 bp PCR product lengths. To screen the polymorphisms and increase the map resolution, CAPS markers were developed based on tomato genome sequences (http://solgenomics.net/).

### Inoculation and disease assay

The pathogenicity of *S. lycopersici* was used for tomato gray leaf spot disease assays according to

the methods of Chen (1996). *S. lycopersici* was collected from Heilongjiang Province and the highly resistant tomato line 'Motelle' and highly susceptible line 'Moneymaker' were used. All 3- to 4-leaf-stage plants were evenly inoculated with a suspension of $1\times10^4$ conidia/ml with a 12 h light period and 85% relative humidity at 25-28℃ (Sun et al., 2016). Disease severity (DS) was rated at 5 days after inoculation with *S. lycopersici* on a scale of 1-5 points (Blansard et al., 1966), and plants with a disease index of 0-1 were resistant; those with a disease index of ≥2 were susceptible.

### Genetic mapping and gene sequence analysis

The linkage mapping software JoinMap4 was used to construct a linkage map with 30-CAPS markers within 1385-$F_2$ mapping individuals (Van et al., 2006). Recombination frequency (rfs) translation and the Kosambi mapping function were used to map genetic distance. Subsequently, the two flanking markers were identified and further to reduce the interval with $F_2$ individuals.

### Gene expression analysis

qRT-PCR was performed to analyze target gene expression levels in the resistant line 'Motelle' and susceptible line 'Moneymaker' post inoculation with *S. lycopersici.* Total RNA was isolated from leaves of resistant and susceptible lines at 0, 24, 48 and 72 hours post inoculation with *S. lycopersici* using TRIzol reagent (Invitrogen, USA) according to the manufacturer's instructions with three biological repeats.

Reverse transcription was conducted using the TaKaRa M-MLV Reverse Transcriptase (RNase H-) reverse transcription kit. The tomato internal reference *EFa1* gene was used in the analyses of gene expression results from qRT-PCR. The relative expression levels of candidate genes were quantified using the $2^{-\Delta CT}$ method.

### Pla*sm*id construction and tomato transformation

The full-length coding region of *R3* (without a termination codon) was amplified from the cDNA by PCR (Table S1), and then the pCAM35::*R3*-GFP fusion construct was inserted into the pCAM35::GFP vector between the KpnI and BamHI sites. The recombinant plasmid pCAM35::*R3*-GFP and the pCAM35::GFP vector were injected into *Nicotiana benthamiana* and then observed with a confocal microscope.

The virus-induced gene silencing (VIGS) technique was used to downregulate the expression of the target gene (André et al., 2009). Tobacco rattle virus (TRV) is the most widely used VIGS vector. It consists of two RNA viral chains, TRV1 and TRV2. The specific sequence of the target gene was amplified by PCR and cloned into the TRV2 vectors, and TRV1 was used to assist the movement of TRV2-containing target gene fragments in plants (Liu et al., 2002). The TRV::*R1*, TRV::*R2*, TRV::*R3*, TRV::00 and TRV::*PDS* vectors were constructed and propagated in LB medium (50 mg mL-1 kanamycin) and then transferred into *A.*

*tumefaciens* strain GV3101 for cell collection from media to an OD 600 of 0.5. Lst. Subsequently, TRV1 and TRV2 were mixed at a ratio of 1:1 and incubated in 3-4-leaf-stage tomato plants of the resistant line Motelle. Ten plants were used for the infiltration of TRV::00, TRV::*PDS* and water, while 150 plants were used for TRV::*R1*, TRV::*R2* and TRV::*R3*. All VIGS plants were grown in a greenhouse at 22-23 °C, and after agroinfiltration for five weeks, the plants were inoculated with *S. lycopersici* in the greenhouse at 25-28 °C. The experiments were performed in three biological replicates.

### Microscopy observations

Trypan blue staining (Wang et al., 2008) and 3,3-diaminobenzidine (DAB) staining (Thordal et al., 1997) as previously described, were used to observe the death of hypersensitive response (HR) cells and the production of $H_2O_2$. The infected leaves were collected at various times after infiltration and were observed under a light microscope.

## Results

### *Sm* is a single dominant resistance gene in tomato

Previous studies have shown that *Sm* is a dominant gene for gray leaf spot resistance. In this study, we chose the resistant tomato line Motelle (Mt, containing the *Sm* gene) and the susceptible line Moneymaker (Mm) as parents to generate an $F_2$ population to map the *Sm* gene. The GLS symptoms in the resistant line Motelle and susceptible line Moneymaker were as shown in Fig. 1A. We performed a disease assay on the $F_2$ population at 5-7 days post inoculation with *S. lycopersici* (Fig. 1B). In the 476 $F_2$ population, 365 were regarded as resistant plants (DS levels between 0 and 1), and 111 were considered susceptible plants with DS levels of 2-5. The ratio of resistant plants to susceptible plants was approximately 3.29:1, which was consistent with the Mendelian segregation ratio of 3:1, as shown in Fig. 1C ($\chi^2 = 0.6302$, $P > 0.05$)This result suggests that the *Sm* gene confers resistance singly via dominant inheritance.

### Fine mapping of *Sm*

A GWAS analysis was first performed and an interval covering *Sm* on Chromosome 11 was identified. A total of 30 CAPS and KASP markers were then developed from this 1.7 Mb interval for fine mapping of the *Sm* gene (Table S2). A total of 1385 $F_2$ individuals were used screen recombinants. Two flanking markers (M29 and M41), whose genetic distances from the *Sm* gene, were 0.6 and 0.3 cM, respectively, were used to identify recombinants (Fig. 2A-B). Based on the analysis of the F2:3 phenotype of 10 recombinants, as shown in Fig. 2, the *Sm* gene was finally mapped between marker M390 and marker M410, and the physical distance was about 160 kb (Fig. 2C-D). As shown in Table 1, a total of 10 genes were predicted in the candidate region (Fig. 2E), according to the tomato genome annotation in SGN website (Table1). However, no nonsynonymous SNPs between the two parental lines was found in the CDS regions of the seven genes (*Solyc11g020000, Solyc11g020003, Solyc11g020007, Solyc11g020030,*

*Solyc11g020040, Solyc11g020050, Solyc11g020060*). Further, no dramatic expression difference of these seven genes was detected between the two parent lines. These results reduced the possibility that the seven genes may be the candidates for *Sm*. Interestingly, we found that three clustered NBS-LRR resistance genes *Solyc11g020080* (named as *R1*), *Solyc11g020090* (named as *R2*) and *Solyc11g020100* (named as *R3*) in this interval. The amino acid lengths of *R1* (126 aa) and *R2* (73 aa) proteins are much shorter than *R3* (819 aa) (Fig. 2F)(Fig. S2A), suggesting that *R1* and *R2* might be pseudogenes. This notion was further supported by the gene expression analysis, as the expression levels of these two genes were much lower than *R3*. More importantly, we didn't identify any nonsynonymous SNPs between the parent lines in R1 and R2, excluding the possibility that they may be *Sm*.

**Table1 Annotated genes in the *Sm* region**

| No. | Gene | Physical position | Functional notes |
|---|---|---|---|
| 1 | *Solyc11g020000* | 10016462… 10017506 | LOW QUALITY:Acyl-CoA N-acyltransferases superfamily protein, putative isoform 2 (AHRD V3.3 *-* A0A061G7B6_THECC) |
| 2 | *Solyc11g020003* | 10024031… 10024797 | Core-2/I-branching beta-1,6-N-acetylglucosaminyltransferase family protein (AHRD V3.3 –* AT1G62305.1) |
| 3 | *Solyc11g020007* | 10024816… 10027512 | LOW QUALITY:Serine/threonine protein phosphatase 7 long form isogeny (AHRD V3.3 *** A0A151TRB0_CAJCA) |
| 4 | *Solyc11g020030* | 10028013… 10028392 | Lysine–tRNA ligase (AHRD V3.3 *-* A0A0V0IK21_SOLCH) |
| 5 | *Solyc11g020040* | 10036469… 10040967 | heat shock protein 70 |
| 6 | *Solyc11g020050* | 10041183… 10041617 | LOW QUALITY:Cytosolic Fe-S cluster assembly factor nar-1 (AHRD V3.3 -** A0A1D1Z971_9ARAE) |
| 7 | *Solyc11g020060* | 10051045… 10058502 | D-3-phosphoglycerate dehydrogenase (AHRD V3.3 *** A0A0B2QT46_GLYSO) |
| 8 | *Solyc11g020080* (*R1*) | 10154490… 10155240 | Disease resistance protein (AHRD V3.3 *-* A0A103Y7*R3*_CYNCS) |
| 9 | *Solyc11g020090* (*R2*) | 10155241… 10155462 | Disease resistance protein (AHRD V3.3 *-* A0A103XRK3_CYNCS) |
| 10 | *Solyc11g0200100* (*R3*) | 10156748… 10161584 | Disease resistance protein (AHRD V3.3 *** A0A118JXS4_CYNCS) |

## *R3* is a candidate gene for *Sm*

We therefore paid more attention on *R3* to verify the candidate gene of *Sm*. We first noticed that the expression of *R3* was significantly induced in response to *S. lycopersici* inoculation, indicating that this

NBS-LRR gene may be involved in the defense response to *S. lycopersici.* Besides, *R3* showed higher expression levels in the resistant line Motelle than that in the susceptible parent line Moneymaker at 48hpi (Fig. 3A). In particular, we found three nonsynonymous SNPs in the CDS region of *R3*, which caused 3 amino acid changes (S394R, E722K and L782I) from Mm to M (Fig. 3B). While the S394R mutation was located at the WHD domain within the NB-ARC region (also known as NBD-HD1-WHD domain), the other two amino acid changes were located at the LRR domain. To investigate the possible roles of these three amino acid mutations, we carefully compared the protein sequences of the resistant (Mt) and susceptible (Mm) versions of *R3* with other NBS-LRR proteins in tomato and *Arabidopsis*. This analysis indicated that the AA394 may be a critical site that affect the function of *R3* and other NBS-LRR proteins. We noticed that, while the Mt version of *R3* and other NBS-LRR proteins have conserved positively charged amino acids (Arginine R or Lysine K ) at AA394, the Mm version of *R3* at this site is serine (S), a non-charged amino acid. This analysis suggest that the K/R at AA 394 might be very critical for NBS-LRR proteins. These results also led us to a hypothesis that the NBS-LRR protein *R3* is non-functional in the cultivated tomatoes due to a critical amino acid mutation, which might be a consequence of selection during tomato domestication. This hypothesis is consistent with the fact that the *Sm*-conferred resistance can be inherited in a dominant manner.

Before further verifying whether *R3* is *Sm*, we analyzed its cellular sub-localization. The *A. tumefaciens* GV3101 strain containing the pCAM35::*R3*-GFP fusion construct was used to infect *N. benthamiana* leaves. The results showed that *R3* was localized to the cytoplasm, nucleus, and cell membrane (Fig. 3C), consistent with previous reports about the cellular sub-localization of NBS-LRRs.

## Functional analysis of *R3*

To further verify *R3* as the underlying gene of *Sm* to confer high resistance to *S. lycopersici,* we thought to downregulate the expression of *R3* in the resistant line Mt to see whether the resistance is abolished or not. VIGS experiments were performed for this purpose. All three clustered NBS-LRR genes *R1, R2* and *R3* were amplified to construct specific TRV::*R1*, TRV::*R2 and* TRV::*R3* VIGS vectors. Three weeks after *agroinfiltration*, the resistant line Mt plants exhibited photobleaching symptoms, demonstrating the silencing was successful. To test whether the gene silencing is specific, expression levels of *R1*, *R2* and *R3* in TRV0 (the vector control), TRV::*R1*, TRV::*R2 and* TRV::*R3* plants were analyzed. As shown in the Fig. 4A, this analysis demonstrated the gene silencing is specific and the silenced plants can be used in further pathogen infection assays.

The TRV::*R1*, TRV::*R2*, TRV::*R3*, and the empty control vector (TRV::00) were inoculated with *S. lycopersici*. As shown in Fig. 4B, no obvious susceptible symptoms were observed on TRV::*R1*, TRV::*R2* and the empty control vector (TRV::00) plants at 3 days post-inoculation (dpi). By contrast, severe disease lesions were observed in TRV::*R3* plants at 3 dpi. Commonly, hypersensitive cell death plays a central role in plant innate immune responses with pathogen restriction. HR (Hypersensitivity reaction) cell death and accumulation of $H_2O_2$ were investigated using trypan blue and DAB(3,3□-diaminobenzidine) staining, respectively. Fig. 4C shows that the HR was impaired and hyphal numbers were enhanced in TRV::*R3*

plants compared with TRV::00 plants at 24 hpi, and the effects were more obvious at 48 hpi. In addition, the accumulation of $H_2O_2$ was lower in the TRV::*R3* plants than in the TRV::00 plants at 24 hpi. Together, these results provide compelling evidence that *R3*, a NBS-LRR resistance protein, is involved in *Sm*-mediated resistance and is the underlying gene of *Sm* for conferring high resistance to *S. lycopersici.*

### *The applicability of CAPS marker in MAS breeding*

The CAPS marker M38 (the position is basically the same as M390), which is closely linked with the Sm gene, was chosen to test whether it can be used for accurately distinguishing resistant and susceptible tomato germplasm materials. As shown by agarose gel electrophoresis in Fig. 5, while homozygous resistant plants had two DNA bands (399 bp and 101 bp) and the homozygous susceptible plants has only one DNA band with 500 bp, the heterozygous plants showed three DNA bands with 399 bp, 101 bp, and 500 bp, respectively. Overall, the CAPS marker M38 showed an accuracy rate of 98.96% when used for evaluating 96 tomato accessions (including resistant and susceptible cultivars). These results indicated that this marker can accurately distinguish resistant and susceptible tomato cultivars and can be used as a molecular marker for MAS breeding.

## Discussion

Recently, tomato gray leaf spot has become a new epidemic disease and is considered a great threat to tomato production worldwide. An effective way to control the disease is to breed resistant varieties in tomato. The *Sm* gene derived from *Solanum pimpinellifolium* was reported to be the only resistance gene, for now, against gray leaf spot disease in tomato. However it has not been cloned yet. In this study, we cloned the *Sm* gene using a map-based cloning strategy. Several lines of evidence support that the *Sm* gene encodes the NBS-LRR plant resistant protein *R3*. First, our fine mapping identified a 160 kb genome region, which contains three NBS-LRR plant resistant genes. Second, we found three nonsynonymous SNPs in the CDS region of *R3*, which caused three amino acid changes (S394R, E722K and L782I) between the Mm and Mt. Third, protein sequence analysis indicated that at least one of the amino acids may be a critical amino acid, which is located at the conserved WHD domain of NBS-LRR proteins. Last, gene silencing of *R3* by VIGS abolished the high resistance in the resistant line Mt, strongly support it is the *Sm* gene. The cloning of *Sm* will be very innovative significance for the utilizing the *Sm* gene in tomato breeding.

To date, an increasing number of NBS-LRR genes conferring resistance to a variety of viruses, bacteria, and fungi have been identified and cloned in plants (Hammond et al., 2003; Martin et al., 2003). NBS-LRR proteins can be divided into two types according to the presence of either an N-terminal coiled-coil domain (CC-NBS-LRR) or Toll-interleukin 1 homology domain (TIR-NBS-LRR) (Meyers et al., 2003). In this study, we found that the *R3* gene encodes a CC-NBS-LRR resistance protein. Although it is a very complicated process, it is well known that the NBS-LRR proteins can induce effector-triggered immunity. Recent structure and functional studies have demonstrated that one NBS-LRR protein *ZAR1*-mediated resistosome is a calcium-permeable channel triggering plant immune signaling. An important direction

for studying the function of *R3* is to test whether it possesses the calcium channel activity for inducing defense response.

An interesting finding in this study is that we identified a critical amino acid change S394R between the resistant and susceptible lines. This amino acid change is located at the WHD domain within the NB-ARC region (also known as NBD-HD1-WHD domain) (Song et al., 2020). Recent structure analysis of the NBS-LRR protein ZAR1 highlighted the importance of the WHD domain. Intriguingly, we noticed that, while the Mt version of *R3* and other NBS-LRR proteins have conserved positively charged amino acids (Arginine R or Lysine K) at 394, Mm version of *R3* at this site is serine (S), a non-charged amino acid. This analysis suggest that the K/R at AA 394 might be very critical for NBS-LRR proteins. Further research on the AA394 will provide critical insights into NBS-LRR functions. These results also suggest that the NBS-LRR protein *R3* may be non-functional in the cultivated tomatoes due to a critical amino acid mutation, which might be a consequence of selection during tomato domestication. This notion is well consistent with the fact that *Sm* confers single dominant resistance to *S. lycopersici.*.

Collectively, based on the results presented in this study, it can be concluded that *Sm* is apparently a typical NLS-LRR gene conferring resistance to gray leaf spot in tomato. More importantly, this finding will accelerate the incorporation of the *Sm* gene into commercial cultivars and provide new opportunities for innovative breeding strategies to breed multi-resistant tomato cultivars.

# Declarations


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### Author contributions

HY, MD and JLdesigned the experiments. ML and JJ performed mapping, gene clone and vector construction. XW performed the transformation of tomato. ZL performed the analysis of GWAS. HY and MD wrote the manuscript. All authors reviewed and approved the final manuscript.

### Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

### Conflict of interest

The authors declare that there is no conflict of interest.

### Supplementary information

The additional files (Table S1, Table S2, Figure S1 and Figure S2).

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# Figures

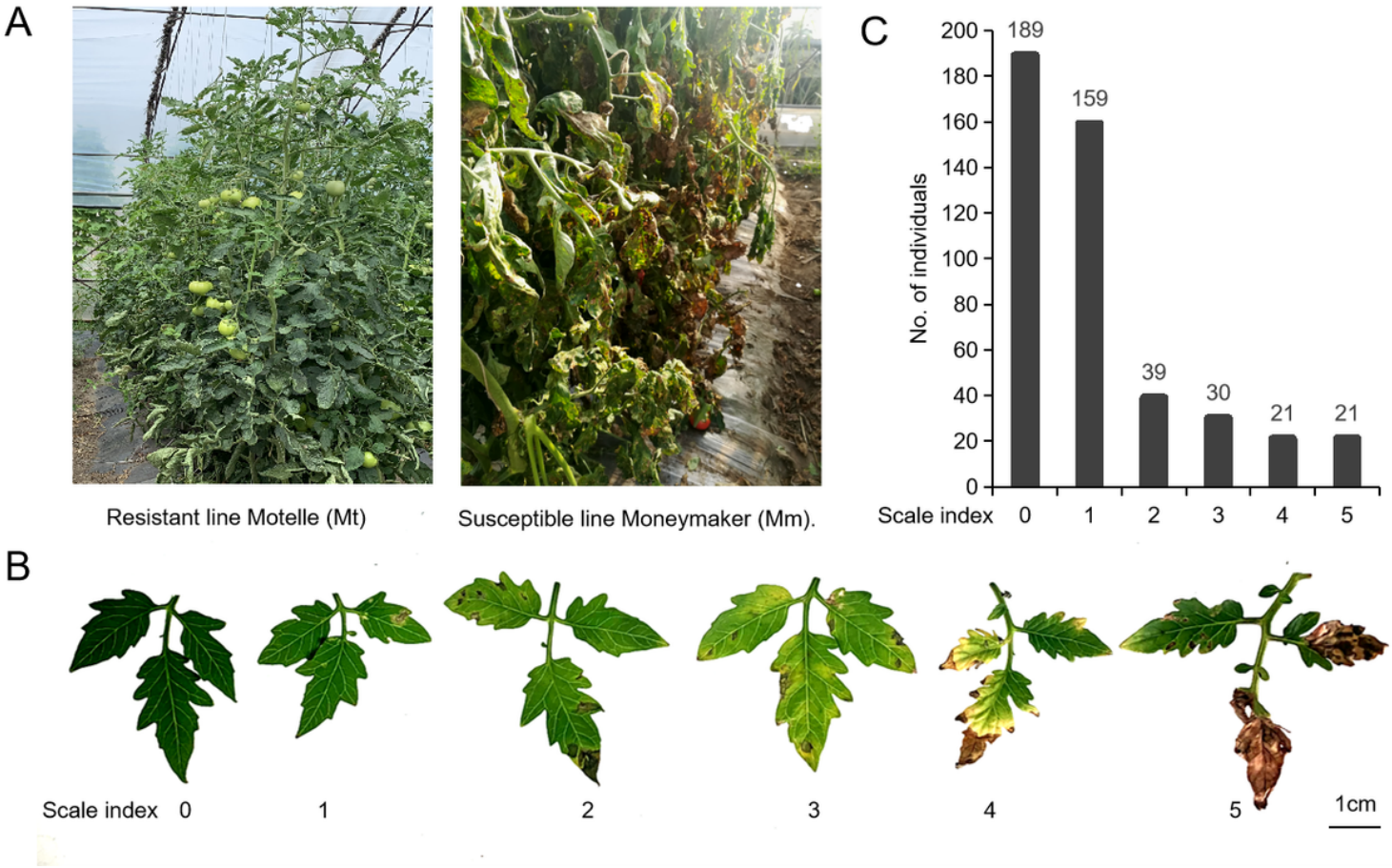
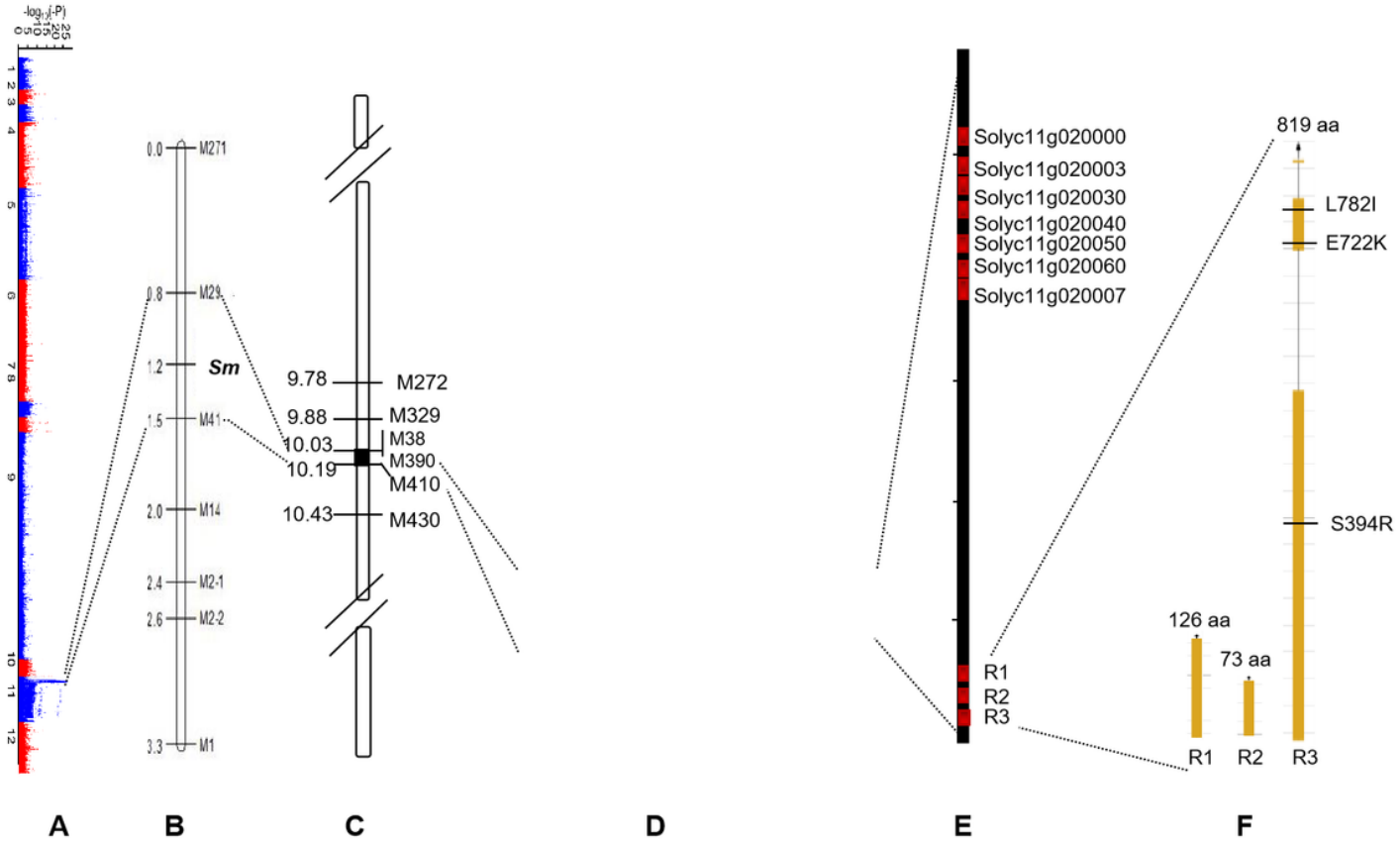


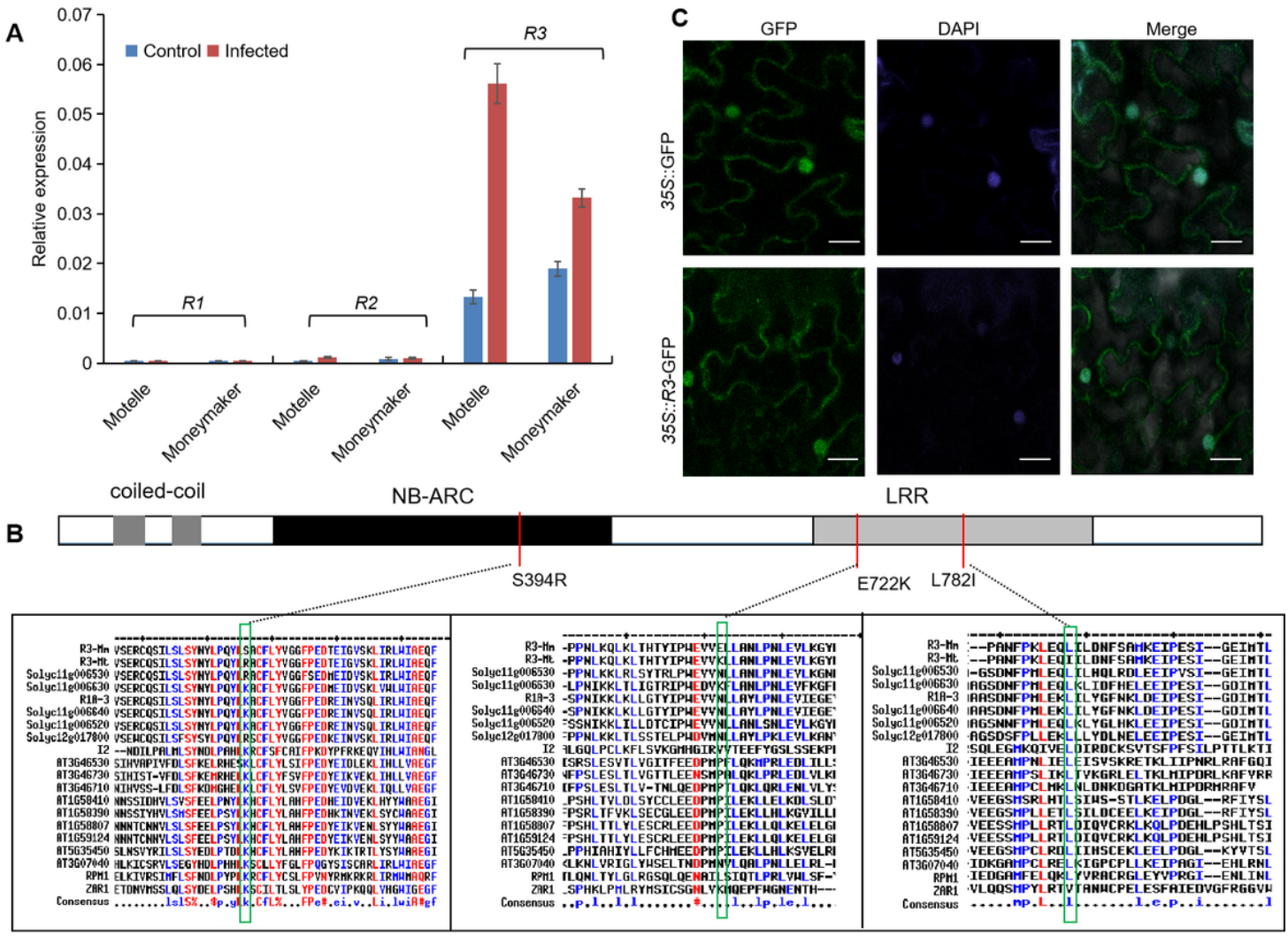

**Figure 1**

Symptoms of GLS disease on tomato plants. A The GLS symptoms in resistant line Motelle and susceptible line Moneymaker. B Symptom rating index (0-5) in the F2 population. C The frequency distribution of the disease scale in the F2 population. The numbers above the bars were the numbers of individuals.



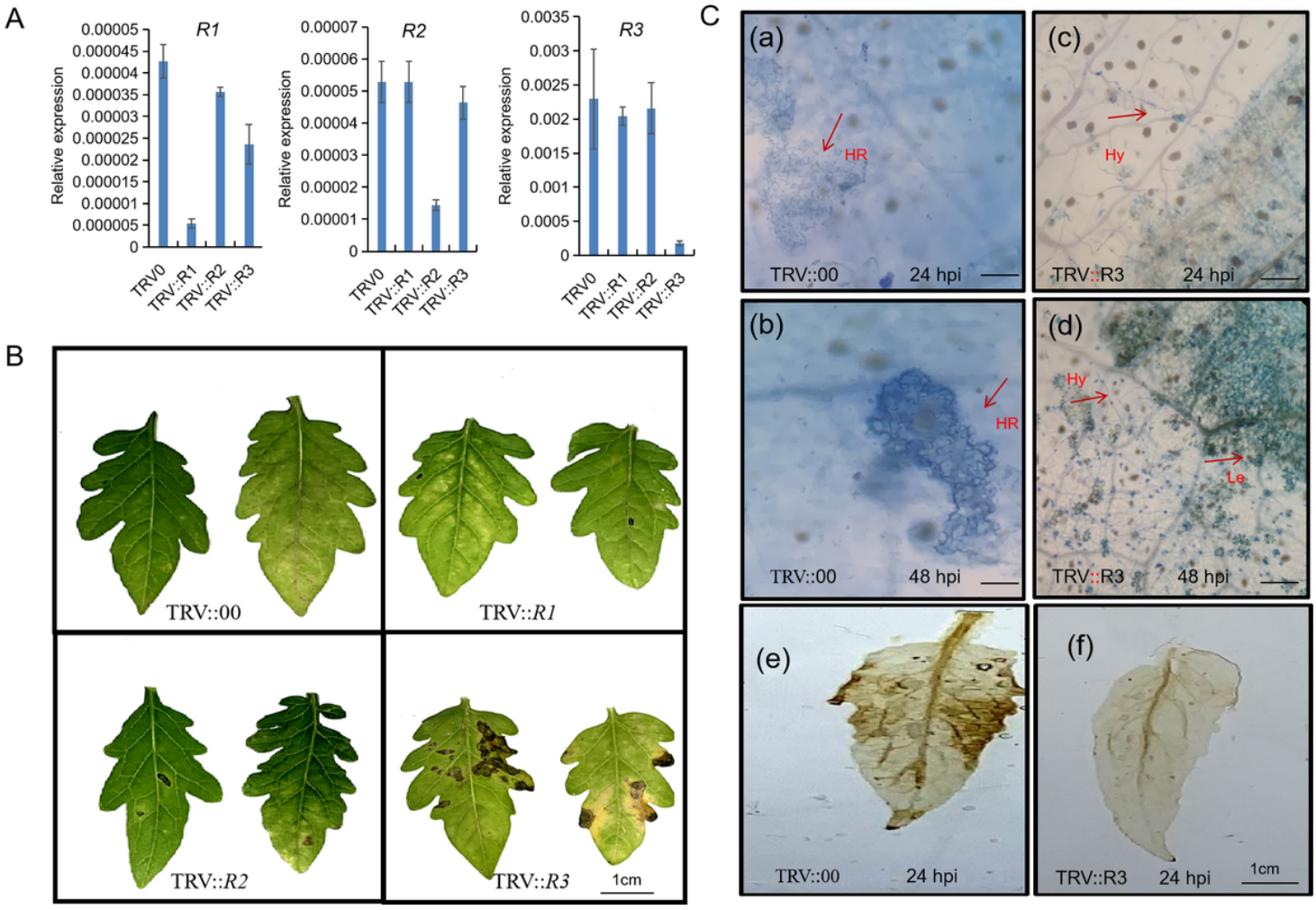

## Figure 2

Genetic and physical maps of the Sm gene. A a Manhattan plot of the GWAS for the Sm gene. B Genetic mapping of Sm. The genetic distances between the two flanking markers M29 and M41, were 0.6 and 0.3 cM, respectively. C-D Sm was mapped to a 160 kb region between M390 and M410 on chromosome 11 based on recombinants. E Ten candidate genes predicted in the region, containing the three clustered NBS-LRR resistance proteins, Solyc11g020080 (R1), Solyc11g020090 (R2) and Solyc11g020100 (R3). F Schematic diagrams of thethree NBS-LRR proteins. S394R: S is changed to R at the amino acid site 394. E722K: E is changed to K at the amino acid site 722.L782I: L is changed to I at the amino acid site 782.




## Figure 3

Relative expression levels of R genes. A R1, R2 and R3 gene expression levels in resistant line Motelle and susceptible line Moneymaker. B Alignment of the predicted protein sequences between R3 and other NBS-LRR proteins. C Subcellular localization of R3. GFP: green fluorescence field, DAPI: 4',6-diamidino-2-phenylindole (DAPI, nuclear staining), Merge: superposition field. Bars=25μm.




## Figure 4

4 Symptoms of silenced plants post-inoculation with S. lycopersici. A The expression levels of R1, R2, R3 in silenced plants; B Disease symptoms of the empty control vector plants (TRV::00) and TRV::R1,TRV::R2 and TRV::R3-silenced plants. C Trypan blue staining of HR-related cell death and the production of H2O2. a-d At 24 hours postinoculation (hpi), no cell death was observed in TRV::R3 plants in contrast with control TRV::00 plants, and the HR was seriously impaired and hyphal growth was strongly enhanced in TRV::R3 plants compared with the TRV::00 plants at 48 hpi. e-f The accumulation of H2O2 was weaker in the TRV::R3 plants than that in the TRV::00 plants at 24 hpi. Hy, hyphae; Le, lesions; HR, hypersensitive response. Bars=50μm

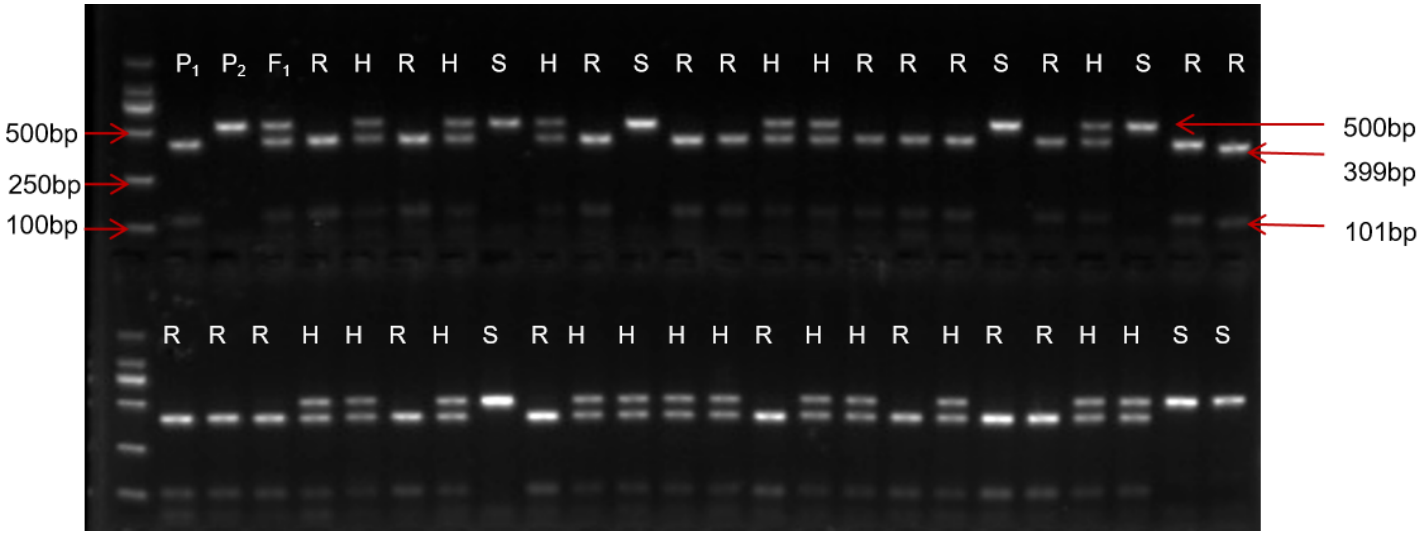



**Figure 5**

Agarose gel electrophoresis of the CAPS marker M38 in tomato germplasm materials. P1: Resistant line Motelle, P2: Susceptible line Moneymaker. R: Phenotype of resistant germplaSm materials, S: phenotype of susceptible germplasm materials.

## Supplementary Files

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