| Primers names | Primers sequence |
|---|---|
| qCML-F | 5'-ATTCTCAGCTCAGGCCAAGA-3' |
| qCML-R | 5'-AACAATTTCAGGGCCACAAT-3' |
| TMV-CP-qPCR-F | 5'-TTAGGTTCCCTGACGGTGAC-3' |
| TMV-CP-qPCR-R | 5'-ACCGTTGCGTCGTCTACTCT-3’ |
| TRV-CP-qPCR-F | 5'-ACTCACGGGCTAACAGTGCT-3' |
| TRV-CP-qPCR-R | 5'-TCTTCCAAAGTCGAGCCAGT-3' |
| TuMV-CP-qPCR-F | 5'-GAATTCGCAGGTGAAACGCTTGAC-3' |
| TuMV-CP-qPCR-R | 5'-GGATCCGTAACCCCTTAACGCCAAG-3' |
| PVX-CP-qPCR-F | 5'-ATTCGCTGCATTCGACTTCT-3' |
| PVX-CP-qPCR-R | 5'-TCTAGGCTGGCAAAGTCGTT-3’ |
| CML-TRV-F BamH I | 5'-CGCGGATCCGACAGCTGATGGATTCAATCAT-3' |
| CML-TRV-R Xhol I | 5'-CCGCTCGAGGCTCTGTTTCGTCGAACGAAT-3' |

**Table 1: Primers used in this study**

**Table 2: Elemental analysis of the SL-gel and CSL-gel.**

| Gel | N (wt%) | C (wt%) | H (wt%) | S (wt%) |
|---|---|---|---|---|
| **SL-gel** | 0 | 10.23 | 8.42 | 0.04 |
| **CSL-gel** | 0.49 | 3.54 | 9.31 | 0.06 |

**Table 3: Kinetic parameters associated with LNT released from the SL-gel and CSL-gel**

| | Higuchi model | | Korsmeyer-Peppas model | | |
|---|---|---|---|---|---|
| **hydrogel** | **$R^2$** | **K** | **$R^2$** | **K** | **n** |
| **SL-gel** | 0.8683 | 0.295 | 0.933 | 0.149 | 0.3089 |
| **CSL-gel** | 0.9823 | 0.447 | 0.989 | 0.229 | 0.6305 |

| Sample | LNT content (1 L water) | Number of deaths | | | |
|---|---|---|---|---|---|
| | | 24h | 48h | 72h | 96h |
| CSL-hydrogel | 50 pieces (LNT:42 mg) | 0 | 0 | 0 | 0 |
| CSL-hydrogel | 100 pieces (LNT:84 mg) | 0 | 0 | 0 | 0 |
| CSL-hydrogel | 200pieces (LNT:168mg) | 0 | 0 | 0 | 0 |
| CSL-hydrogel | 400 pieces (LNT:336mg) | 0 | 1 | 1 | 1 |

**Table 4: Toxicity of CSL-gel to Crucian Carp.**

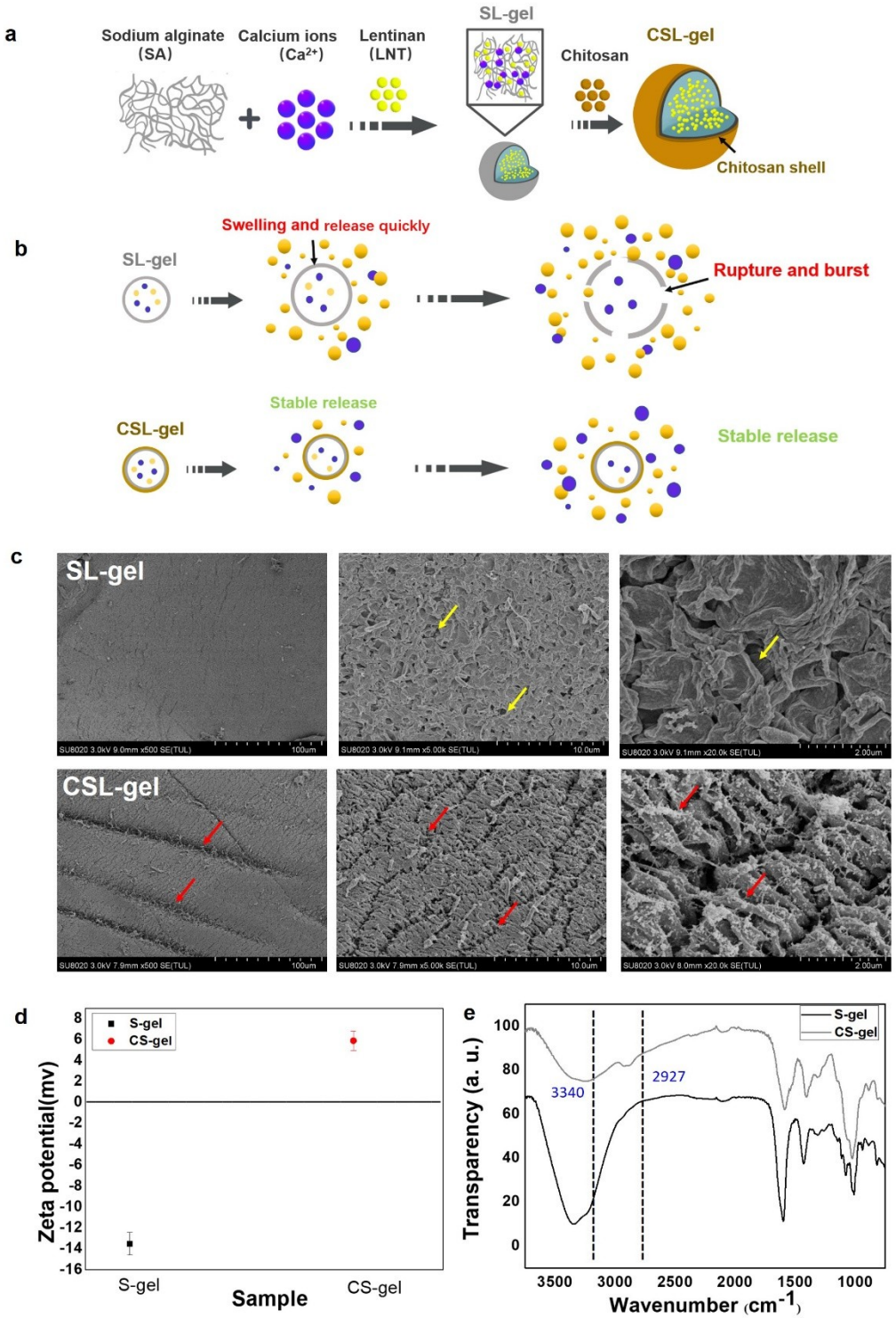


**Figure 1. Synthesis of CSL-gel and characterization of chitosan shell**. (a). schematic representation of the synthesis of CSL-gel. (b). Diagram of CSL-gel and SL-gel drug release simulation. (c). SEM images of surface composition of SL-gel and CSL-gel. (d). Zeta potential of the S-gel without LNT and chitosan shell and CS-gel without LNT. (e). FTIR spectra of the S-gel and CS-gel.

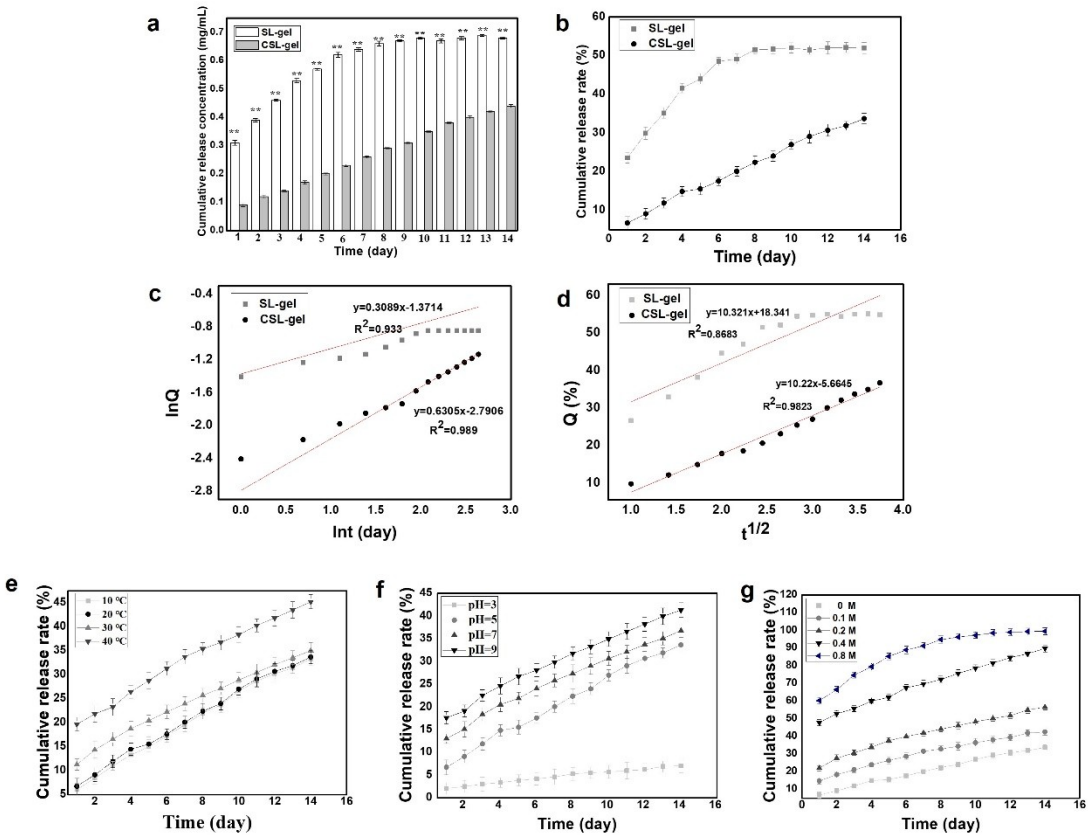


**Figure 2. CSL-gel exhibits sustainable and cumulative release of LNT**. (a). Comparison of the cumulative release of LNT from the SL-gel and CSL-gel. (b). The cumulative release rate of LNT from CSL-gel and SL-gel. (c). The fitting curves for the Korsmeyer-Peppas model. (d). The fitting curves for the Higuchi model. (e). The cumulative release rate of LNT from CSL-gel at different temperatures. (f), The cumulative release rate of LNT from CSL-gel at different pH. (g). The cumulative release rate of LNT from CSL-gel at different $Na^+$ concentration. Double asterisks indicate separation among CSL-gel and SL-gel at the same amount by Duncan's multiple comparisons (**, $p < 0.01$). Vertical bars indicate standard deviations ($n = 3$).

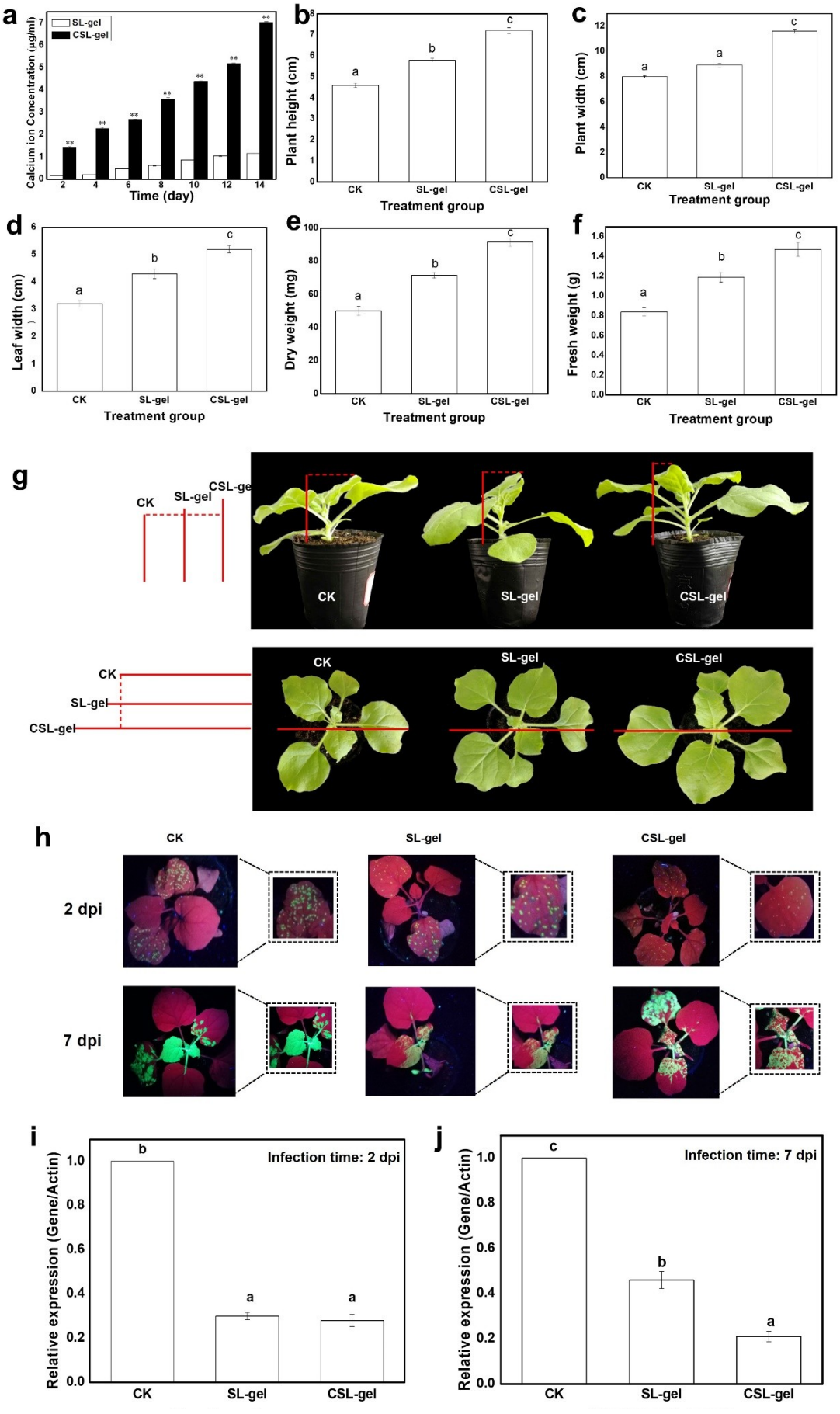
a
SL-gel
CSL-gel
Calcium ion Concentration (μg/ml)
Time (day)
b
Plant height (cm)
Treatment group
CK
SL-gel
CSL-gel
c
Plant width (cm)
d
Leaf width (cm)
e
Dry weight (mg)
f
Fresh weight (g)
g
h
2 dpi
7 dpi
i
Infection time: 2 dpi
Relative expression (Gene/Actin)
j
Infection time: 7 dpi

**Figure 3. CSL-gel with the sustainable release of calcium ions promotes plant growth and significantly enhances the resistance of *N. benthamiana* against TMV**. (a). Comparison of calcium ions cumulative release between SL-gel and CSL-gel. (b-f).Comparison of plant height, plant width, leaf width, dry weight, and fresh weight between CSL-gel and SL-treated plants. (g). Representative pictures showing the plant growth between different treatments. (h). CSL-gel treated *N. benthamiana* plants display enhanced resistance against TMV. *N. benthamiana* plants were inoculated with TMV–GFP constructs by rubbing and representative pictures were photographed at 2 and 7 dpi. (i) qPCR analysis showing the expression level of *TMV−CP* in the inoculated leaves of *N. benthamiana* at 2 dpi (j). qPCR analysis showing the expression level of TMV−CP in the inoculated leaves of *N. benthamiana* at 7 dpi. The expression level was normalized to Actin. Mean values displayed in each bar followed by different letters, are significantly different according to Duncan's multiple range test ($p < 0.05$).

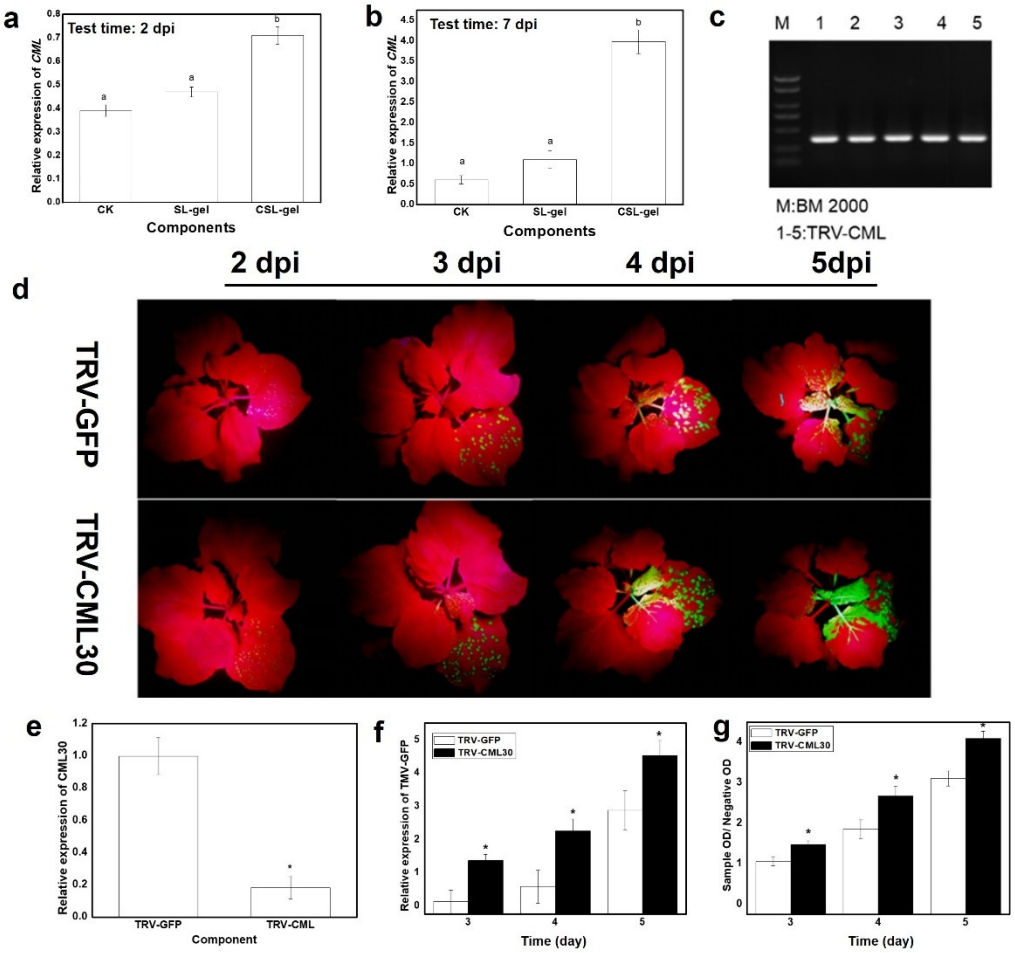


**Figure 4. CSL-gel triggers *CML30* expression and silencing *CML30* increases TMV infection in *N. benthamiana***. (a-b). CSL-gel treatment triggers the expression of *CML30* at 2 days and 7 days. (c). The PCR results showing the presence of recombinant plasmids in Agrobacterium. (d). Silencing *CML30* increases TMV infection. The leaves of *N. benthamiana* were inoculated with TMV−GFP by rubbing.The green GFP fluorescence signals were visualized at 2, 3, 4 and 5 dpi under UV light. Representative pictures are showed. (e). qPCR analysis showing the relative expression of *CML30* in the silenced *N. benthamiana*. (f). qPCR analysis showed the expression level of TMV−CP in the inoculated leaves of *CML30* silenced *N. benthamiana* at 3, 4 and 5 dpi. (g). The accumulation of TMV-CP protein was detected by ELISA.

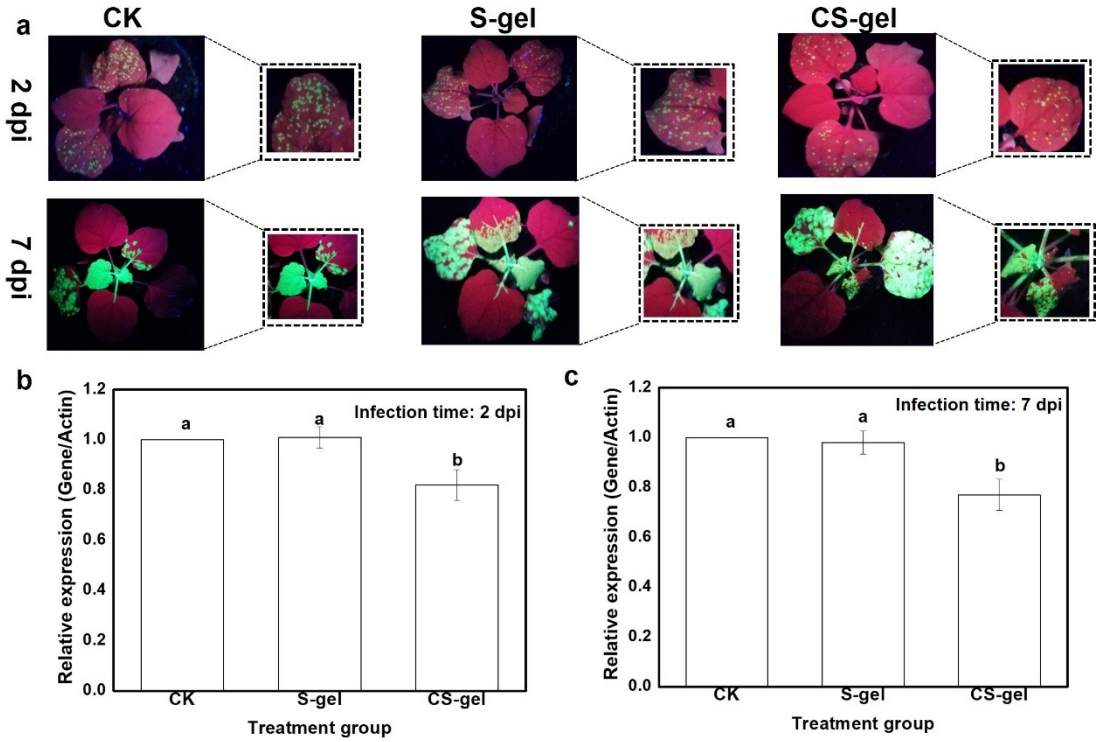
a
CK
S-gel
CS-gel
2 dpi
7 dpi
b
Relative expression (Gene/Actin)
Infection time: 2 dpi
a
a
b
CK
S-gel
CS-gel
Treatment group
c
Relative expression (Gene/Actin)
Infection time: 7 dpi
a
a
b
CK
S-gel
CS-gel
Treatment group

**Figure 5. Comparison of the anti-TMV activity of S-gel and CS-gel.** (a). *N. benthamiana* plants treated with CS-gel display increased resistance against TMV. The leaves of *N. benthamiana* were inoculated with TMV−GFP by rubbing. The green GFP fluorescence signals were visualized at 2 and 7 dpi under UV light. Representative pictures are showed. (b). qPCR analysis showing the relative expression of *TMV-CP* in the inoculated leaves of *N. benthamiana* at 2 dpi. (c). qPCR analysis showed the expression level of *TMV−CP* in the young leaves of *N. benthamiana* at 7 dpi.

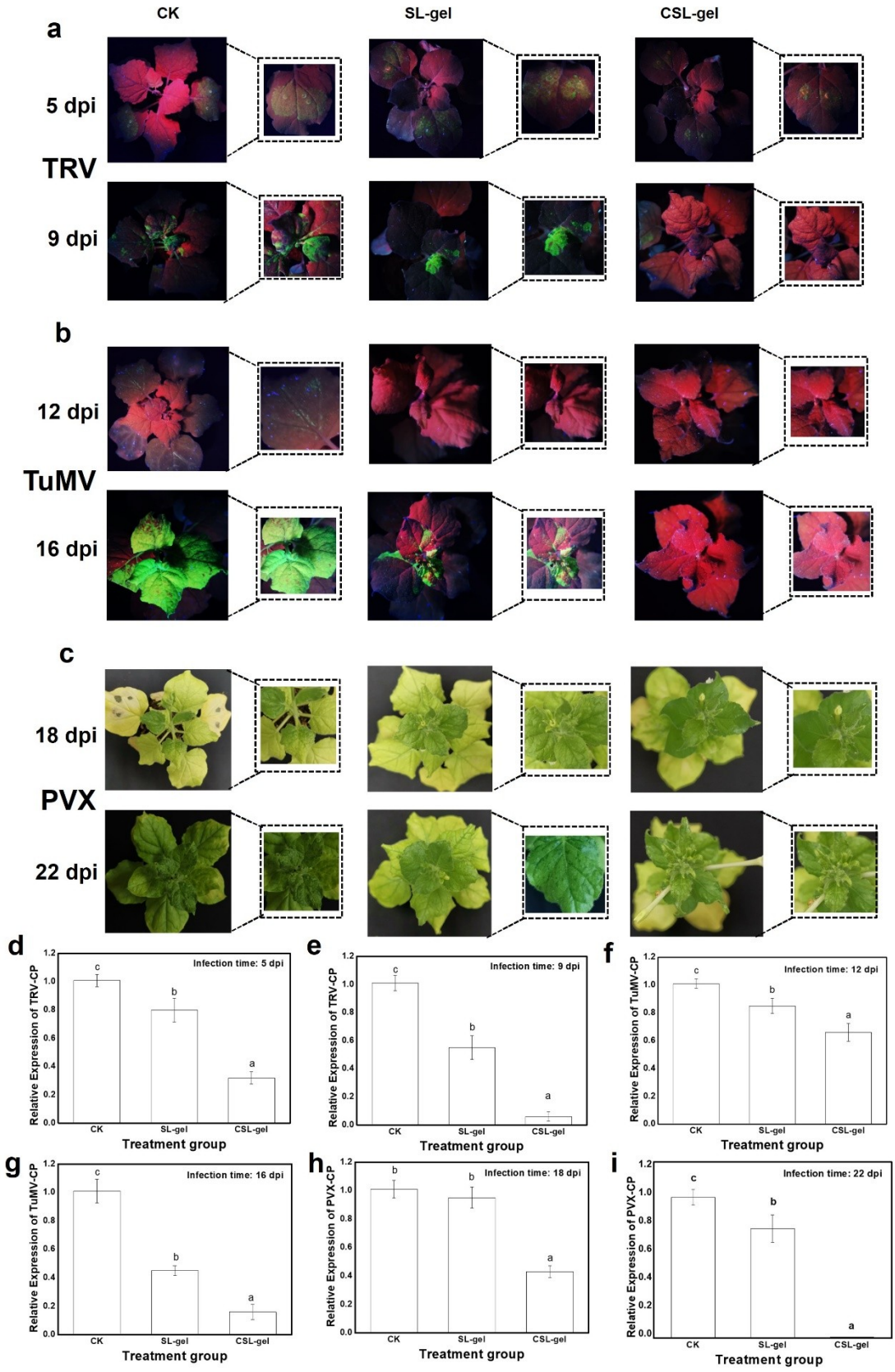
CK
SL-gel
CSL-gel
a
5 dpi
TRV
9 dpi
b
12 dpi
TuMV
16 dpi
c
18 dpi
PVX
22 dpi
d
Infection time: 5 dpi
Relative Expression of TRV-CP
e
Infection time: 9 dpi
Relative Expression of TRV-CP
f
Infection time: 12 dpi
Relative Expression of TuMV-CP
g
Infection time: 16 dpi
Relative Expression of TuMV-CP
h
Infection time: 18 dpi
Relative Expression of PVX-CP
i
Infection time: 22 dpi
Relative Expression of PVX-CP
CK
SL-gel
CSL-gel
Treatment group

**Figure 6. CSL-gel treated *N. benthamiana* plants exhibit significantly increased resistance against TRV, TuMV and PVX**. The leaves of *N. benthamiana* were inoculated with TRV, TuMV and PVX by rubbing or agroinfiltration 14 days after CSL-gel treatment. (a). The green GFP fluorescence signals were visualized at 5 and 9 days after TRV inoculation under UV light. Representative pictures are showed. (b). The green GFP fluorescence signals were visualized at 12 and 16 days after TuMV inoculation under UV light. Representative pictures are showed. (c). Disease symptoms caused by PVX were observed at 18 and 22 days after inoculation and representative pictures are showed. (d-e). qPCR analysis showing the relative expression of *TRV-CP* in the inoculated leaves of *N. benthamiana* at 5 dpi and in the young leaves at 9 dpi. (f-g). qPCR analysis showing the relative expression of T*uMVV-CP* in the inoculated leaves of *N. benthamiana* at 12 dpi and in the young leaves at 16 dpi. (h-i). qPCR analysis showing the relative expression of *PVX-CP* in the inoculated leaves of *N. benthamiana* at 18 dpi and in the young leaves at 22 dpi.

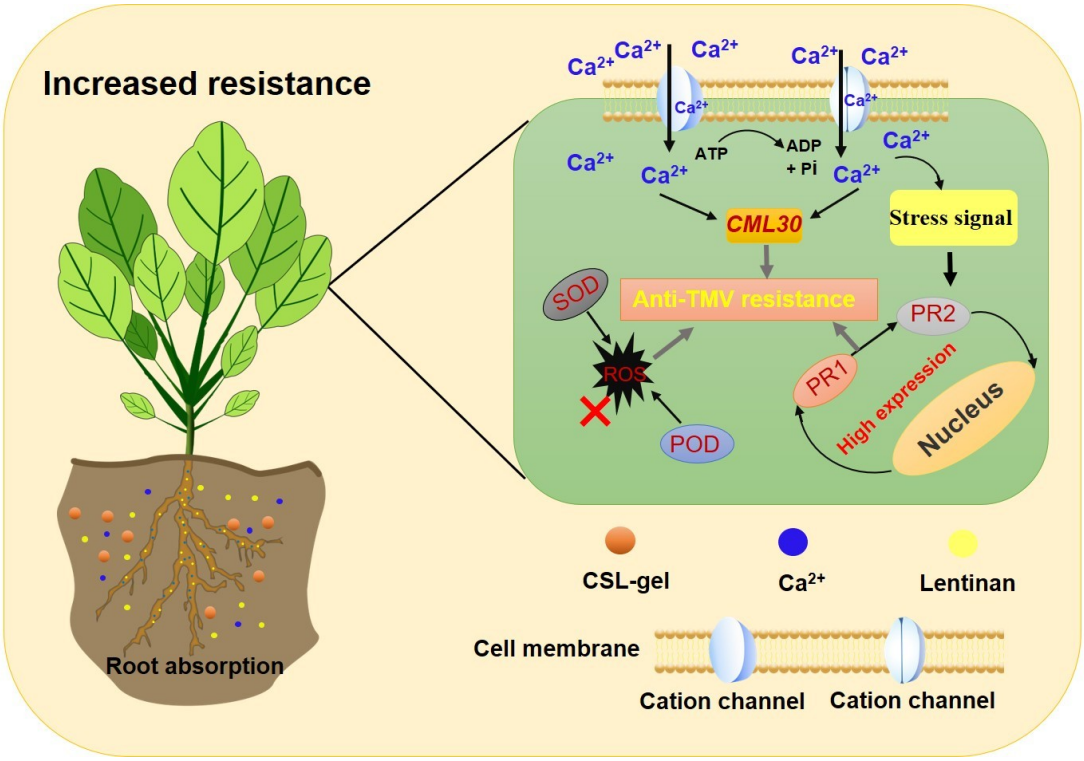


**Figure 7.** Schematic representation of a proposed action model of CSL-gel on plants to improve broad-spectrum resistance to different viruses.