Table S1. Primers used in this study.

Primer name	Sequence	Primer use	Position of primers in gene sequence
Rscb-S1	5′-CACTCCAATGGCTCTGTTCC-3′	RT-PCR	nt.11-30 of GU360972
Rscb-A1	5′-GCAAAAGGAACAAGCAAACAT-3′		nt.1163-1183 of GU360972
ISHS- T7S1[a]	5′-GGATCCTAATACGACTCACTATAGGGGTCCTCTGCCTCCGTGAT-3′	ISH template	nt.437-454 of GU360972
ISHS- A	5′- TGCCGAAGTGCTTGTCC-3′		nt.737-753 of GU360972
ISHA- S2	5′- GTCCTCTGCCTCCGTGAT-3′	ISH template	nt.437-454 of GU360972
ISHA- T7A2[a]	5′-GGATCCTAATACGACTCACTATAGGGTGCCGAAGTGCTTGTCC -3′		nt.737-753 of GU360972
qPCR-F1	5′-CGCAAGCAGTTCACCAAGT-3′	qRT-PCR	nt.360-378 of GU360972
qPCR-R1	5′-GGATGTCCTGTGCCGAGA-3′		nt.502-519 of GU360972
Actin-F	5′-GAAAGAGGGCCGGAAGAG-3″	qRT-PCR	nt.204-221of EU000540
Actin-R	5′-AGATCGTCCGCGACATAAAG-3′		nt.43-62 of EU000540
Pe-F	5′- GAGAGA GGATCCATGGCTCTGTTCCGCCTG -3′	Prokaryotic expression	nt.18-35of GU360972
Pe-R	5′- GAGAGACTCGAGCTCAACGATCGCTGACGC -3′		nt.1068-1085 of GU360972
Rscb-T7S[a]	5′-GGATCCTAATACGACTCACTATAGGGGCTCTGTTCCGCCTGTGC-3′	dsRNA template	nt.21-38 of GU360972
Rscb-A	5′-ACCGTTGCATCCCTGGCT-3′		nt.540-557 of GU360972
Rscb-S	5′- GCTCTGTTCCGCCTGTGC-3′	dsRNA template	nt.21-38 of GU360972
Rscb-T7A[a]	5′-GGATCCTAATACGACTCACTATAGGGACCGTTGCATCCCTGGCT -3′		nt.540-557 of GU360972
eGFP-T7S[a]	5′-GGATCCTAATACGACTCACTATAGGGCAGTGCTTCAGCCGCTACC-3′	dsRNA template	nt.208-226 of DQ768212
eGFP-A	5′-AGTTCACCTTGATGCCGTTCTT-3′		nt.475-496 of DQ768212
eGFP-S	5′-CAGTGCTTCAGCCGCTACC-3′	dsRNA template	nt.208-227 of DQ768212
eGFP-T7A[a]	5′-GGATCCTAATACGACTCACTATAGGGAGTTCACCTTGATGCCGTTCTT -3′		nt.475-496 of DQ768212
RNAi-F	5′-CCGCTCGAGTCTAGAGACTCCCGCAAGCAGTTCAC-3′	Vector construction	nt.354-373 of GU360972
RNAi-R	5′-CTAGCCATGGATCCTCGCATTTCTTCGAGCATTCC-3′		nt.686-706 of GU360972
eGFP-F	5′-CCGCTCGAGTCTAGATGCTTCAGCCGCTACCC-3′	Vector construction	nt.211-227 of DQ768212
eGFP-R	5′-CATGCCATGGATCC AGTTCACCTTGATGCCGTTC-3′		nt.477-496 of DQ768212
CHSA-F	5′-ACTTGCCTTGGAGTTTATGTT-3′	PCR detection	nt.4057-4077 of AY310901
OCS-R	5′-TTGTTATTGTGGCGCTCTATC-3′		nt.4443-4463 of AY310901
RNAi-F1	5′-GACTCCCGCAAGCAGTTCAC-3′	Southern blot	nt.354-373 of GU360972
RNAi-R1	5′-TCGCATTTCTTCGAGCATTCC-3′		nt.686-706 of GU360972

[a] The T7 promoter sequence is underline

Supplementary Figures Captions

Figure S1. Agarose gel electrophoresis of PCR products and the synthetic dsRNA. (A) PCR products of full-length *Rs-cb-1* gene from *Radopholus similis*. **(B)** PCR products of sense (Lane 1) and antisense (Lane 2) strand templats of *Rs-cb-1* amplified from *R. similis*. **(C)** PCR products of sense (Lane 1) and antisense (Lane 2) strand templats of e*gfp* amplified from the vector of PYL 322-d1- e*gfp*n. **(D)** dsRNA synthesis of *Rs-cb-1*. **(E)** dsRNA synthesis of e*gfp*. M, DNA marker (DL2000).

Figure S2. RNAi effect of *Rs-cb-1* on embryonic development of *Radopholus similis*. Average time of embryonic development **(A)** and average hatching rate of the second stage juvenile **(B)** of *R. similis* after treated with *Rs-cb-1* dsRNA. **(C, D)** Average percentage of eggs in different embryonic development stages after treated with *Rs-cb-1* dsRNA. **(C)** Cultured for 7days after RNAi. **(D)** Cultured for 11days after RNAi. CK, untreated eggs; G12, G24, G36, G48 and G72, expression of eggs treated with non-endogenous e*gfp* dsRNA for 12 h, 24 h, 36 h, 48 h and 72 h, respectively; R12, R24, R36, R48 and R72, expression of eggs treated with *Rs-cb-1* dsRNA for 12 h, 24 h, 36 h, 48 h and 72 h, respectively. *Bars* indicate standard errors of mean data (n=5) and different letters indicate significant differences (p<0.05) among different treatments.

Figure S3. Average times of post-embryonic development of *R. similis* after treated with *Rs-cb-1* dsRNA. CK, untreated eggs; G12, G24, G36, G48 and G72, expression of eggs treated with non-endogenous e*gfp* dsRNA for 12 h, 24 h, 36 h, 48 h and 72 h, respectively; R12, R24, R36, R48 and R72, expression of eggs treated with *Rs-cb-1* dsRNA for 12 h, 24 h, 36 h, 48 h and 72 h, respectively. *Bars* indicate standard errors of mean data (n=5) and different letters indicate significant differences (p<0.05) among different treatments.

Figure S4. Infection symptoms of *Anthurium andraeanum* after being inoculated with *Radopholus similis* for 2 months. (A) R12-R72, inoculated nematodes treated with *Rs-cb-1* dsRNA for 12 h, 24 h, 36 h, 48 h and 72 h, respectively. **(B)** G12-G72, inoculated nematodes treated with non-endogenous e*gfp* dsRNA for 12 h, 24 h, 36 h, 48 h and 72 h, respectively. **(C)** M12-M72, inoculated nematodes treated with M9 buffer for 12 h, 24 h, 36 h, 48 h and 72 h, respectively. **(D)** N, inoculated nematodes

treated with nothing; W12-W72, inoculated nematodes treated with water for 12 h, 24 h, 36 h, 48 h and 72 h, respectively. CK, uninoculated control. The roots corresponding to the above plants, respectively.

Figure S5. Pathogenicity of *Radopholus similis* to *Anthurium andraeanum* is decreased significantly after soaking with *Rs-cb-1* dsRNA. Plant height **(A)**, fresh shoot weight **(B)**, fresh root weight **(C)** and the number of nematodes in the rhizosphere **(D)** of *A. andraeanum* after being inoculated with different treatments of *R. similis* for 2 months. CK, uninoculated control; W, inoculation of untreated nematodes; R12, R24, R36, R48 and R72, inoculation of nematodes treated with *Rs-cb-1* dsRNA for 12 h, 24 h, 36 h, 48 h and 72 h, respectively; G12, G24, G36, G48 and G72, inoculation of nematodes treated with non-endogenous e*gfp* dsRNA for 12 h, 24 h, 36 h, 48 h and 72 h, respectively. *Bars* indicate standard errors of mean data (n=5) and different letters indicate significant differences (p <0.05) among different treatments.

Figure S6. The growth morphology of tobacco leaves. 1, 2, 3 and 4, expression of wild type, *Rs-cb-1* transgenic, e*gfp* transgenic and empty transformation vector tobacco leaves, respectively.

Figure S7. Genetic stability analysis of T1 generation *Rs-cb-1* transgenic plants. (A-C) Expression of genomic DNA from the No. 2, 3 and 6 *Rs-cb-1* transgenic lines, respectively. M, DNA marker (DL2000); 1-15, independent T1 generation *Rs-cb-1* transgenic lines from the same T0 generation transgenic tobacco seeds.

Figure S8. Resistance to *Radopholus similis* is improved in T2 generation transgenic tobacco plants expressing *Rs-cb-1* dsRNA. Plant height **(A)**, fresh shoot weight **(B)**, fresh root weight **(C)**, the number of nematodes in the rhizosphere **(D)** and per gram root **(E)** and the root infection symptoms **(F)** of different tobacco plants after being inoculated with 2000 nematodes for 75 days. Nematodes in different treatments of tobacco roots were stained with acid fuchsin. *Bars* indicate standard errors of mean data (n=5). **(G)** *Rs-cb-1* mRNA levels in *R. similis* are significantly suppressed by feeding with *Rs-cb-1* dsRNA transgenic plants. *Bars* indicate standard errors of mean data (n=3) and different letters indicate significant differences (p<0.05) among different treatments. CK, uninoculated wild-type tobacco plants; cb, *Rs-cb-1* transgenic plants; e*gfp*, e*gfp* transgenic plants; pFGC, empty transformation

vector plants; WT, wild-type tobacco plants.

Figure S9. Persistence and inheritance of *Rs-cb-1* gene silencing induced by RNAi. The recovery of *Rs-cb-1* expression at different times in *Radopholus similis* after soaked with *Rs-cb-1* dsRNA for 48 h **(A)** or isolated from T2 generation *Rs-cb-1* transgenic tobacco roots **(B)**. 1-15, expression of nematodes were maintained in sterile water and sampled at 1, 3, 5, 7, 9, 11, 13 and 15 days post treatment, respectively. qPCR assays for the expression levels of *Rs-cb-1* in F1 generation **(C)** and F2 generation **(I)** nematodes. *Bars* indicate standard errors of mean data (n=3). **(D)** Number of F1 generation nematodes on carrot callus 30 days after inoculation of 30 females, respectively. Plant height **(E)**, fresh shoot weight **(F)**, fresh root weight **(G)** and the number of nematodes in the rhizosphere **(H)** of tobacco plants after being inoculated with 400 F1 generation nematodes for 45 days. CK, untreated control nematodes; dsRNA, nematodes treated with *Rs-cb-1* dsRNA for 48 h; Tobacco, nematodes collected from T2 generation *Rs-cb-1* transgenic tobacco roots. *Bars* indicate standard errors of mean data (n=5) and different letters indicate significant differences (p<0.05) among different treatments.

Figure S1

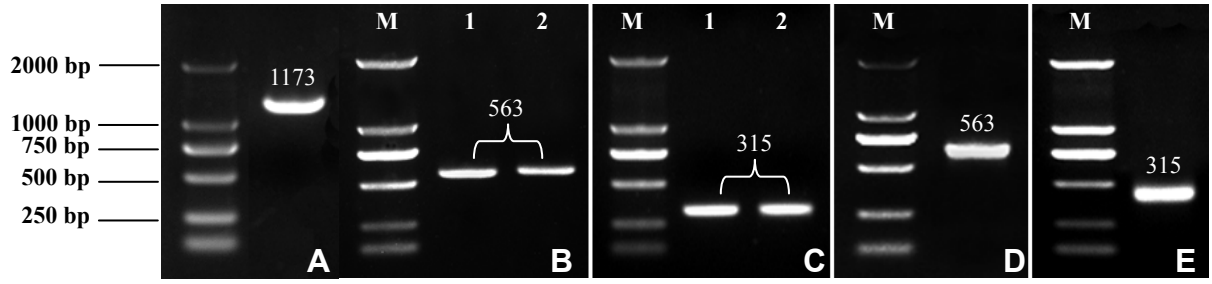


Figure S2

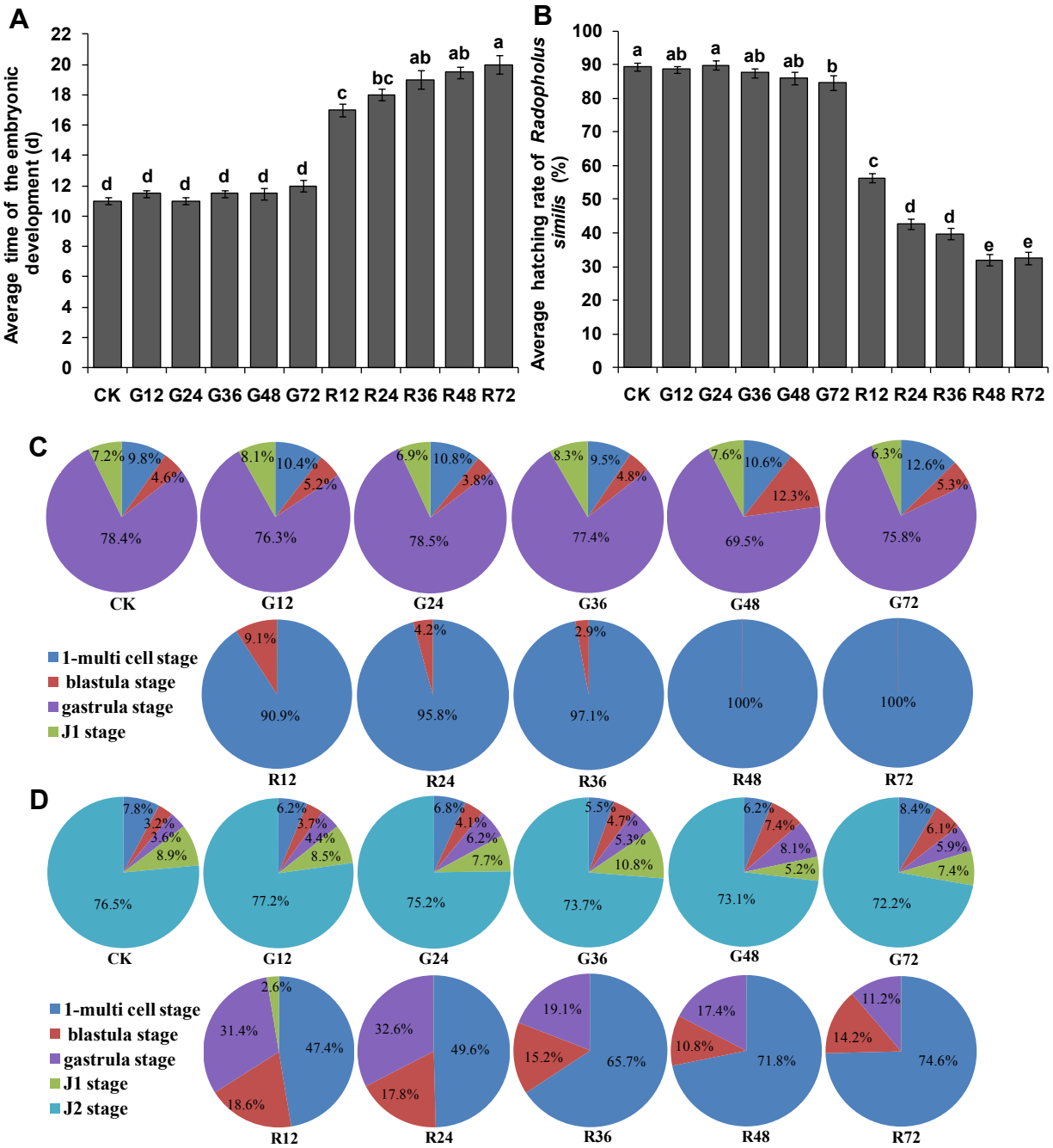

A
Average time of the embryonic development (d)
0
2
4
6
8
10
12
14
16
18
20
22
d d d d d d c bc ab ab a
CK G12 G24 G36 G48 G72 R12 R24 R36 R48 R72
B
Average hatching rate of Radopholus similis (%)
0
10
20
30
40
50
60
70
80
90
100
a ab a ab ab b c d d e e
CK G12 G24 G36 G48 G72 R12 R24 R36 R48 R72
C
7.2% 9.8% 4.6% 78.4%
8.1% 10.4% 5.2% 76.3%
6.9% 10.8% 3.8% 78.5%
8.3% 9.5% 4.8% 77.4%
7.6% 10.6% 12.3% 69.5%
6.3% 12.6% 5.3% 75.8%
CK G12 G24 G36 G48 G72
1-multi cell stage
blastula stage
gastrula stage
J1 stage
9.1% 90.9%
4.2% 95.8%
2.9% 97.1%
100%
100%
R12 R24 R36 R48 R72
D
7.8% 3.2% 3.6% 8.9% 76.5%
6.2% 3.7% 4.4% 8.5% 77.2%
6.8% 4.1% 6.2% 7.7% 75.2%
5.5% 4.7% 5.3% 10.8% 73.7%
6.2% 7.4% 8.1% 5.2% 73.1%
8.4% 6.1% 5.9% 7.4% 72.2%
CK G12 G24 G36 G48 G72
1-multi cell stage
blastula stage
gastrula stage
J1 stage
J2 stage
2.6% 31.4% 47.4% 18.6%
32.6% 49.6% 17.8%
19.1% 15.2% 65.7%
17.4% 10.8% 71.8%
11.2% 14.2% 74.6%
R12 R24 R36 R48 R72

Figure S3

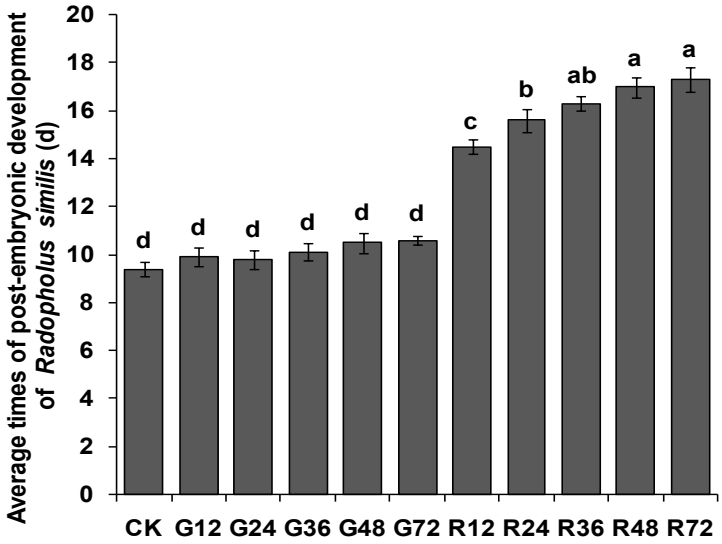

Average times of post-embryonic development of *Radopholus similis* (d)
20
18
16
14
12
10
8
6
4
2
0
d
d
d
d
d
d
c
b
ab
a
a
CK
G12
G24
G36
G48
G72
R12
R24
R36
R48
R72

Figure S4

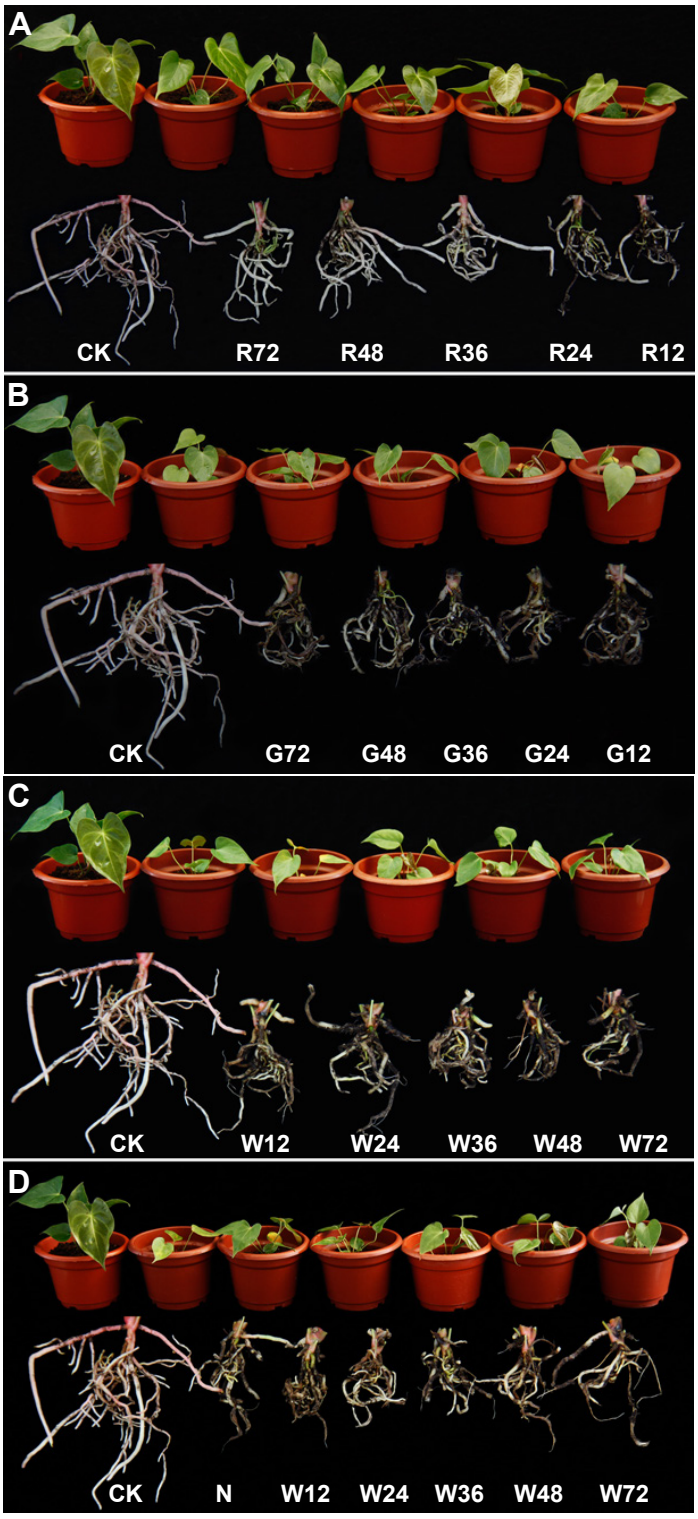

A
CK
R72
R48
R36
R24
R12
B
CK
G72
G48
G36
G24
G12
C
CK
W12
W24
W36
W48
W72
D
CK
N
W12
W24
W36
W48
W72

Figure S5

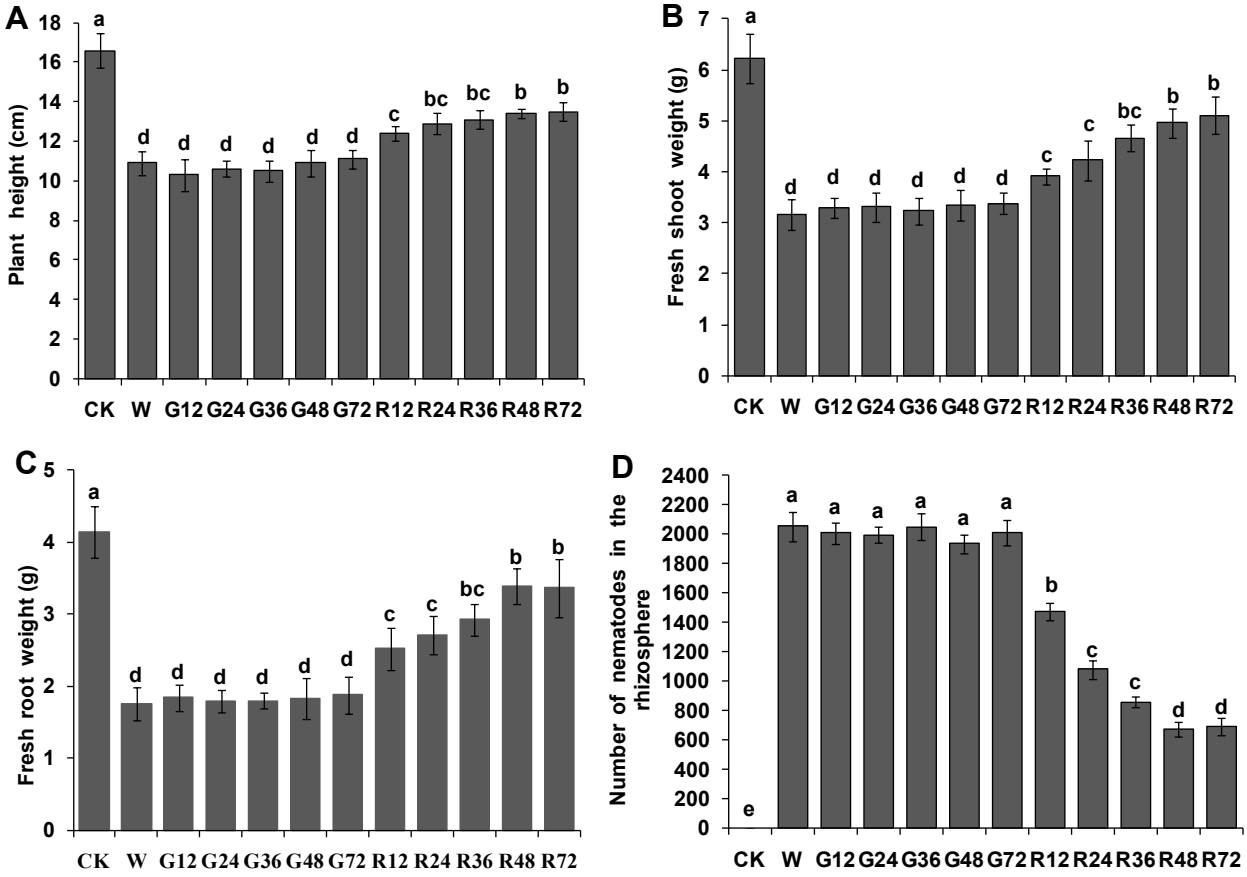

A
Plant height (cm)
a d d d d d d c bc bc b b
B
Fresh shoot weight (g)
a d d d d d d c c bc b b
C
Fresh root weight (g)
a d d d d d d c c bc b b
D
Number of nematodes in the rhizosphere
e a a a a a a b c c d d
CK W G12 G24 G36 G48 G72 R12 R24 R36 R48 R72

Figure S6

Figure S7

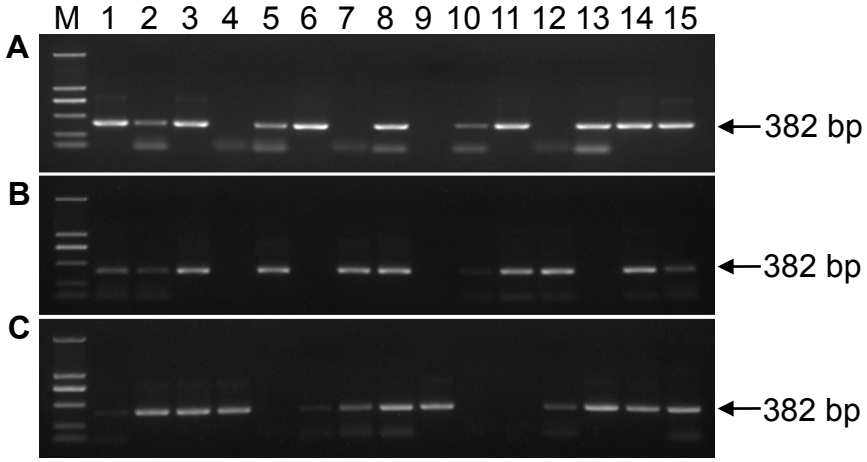
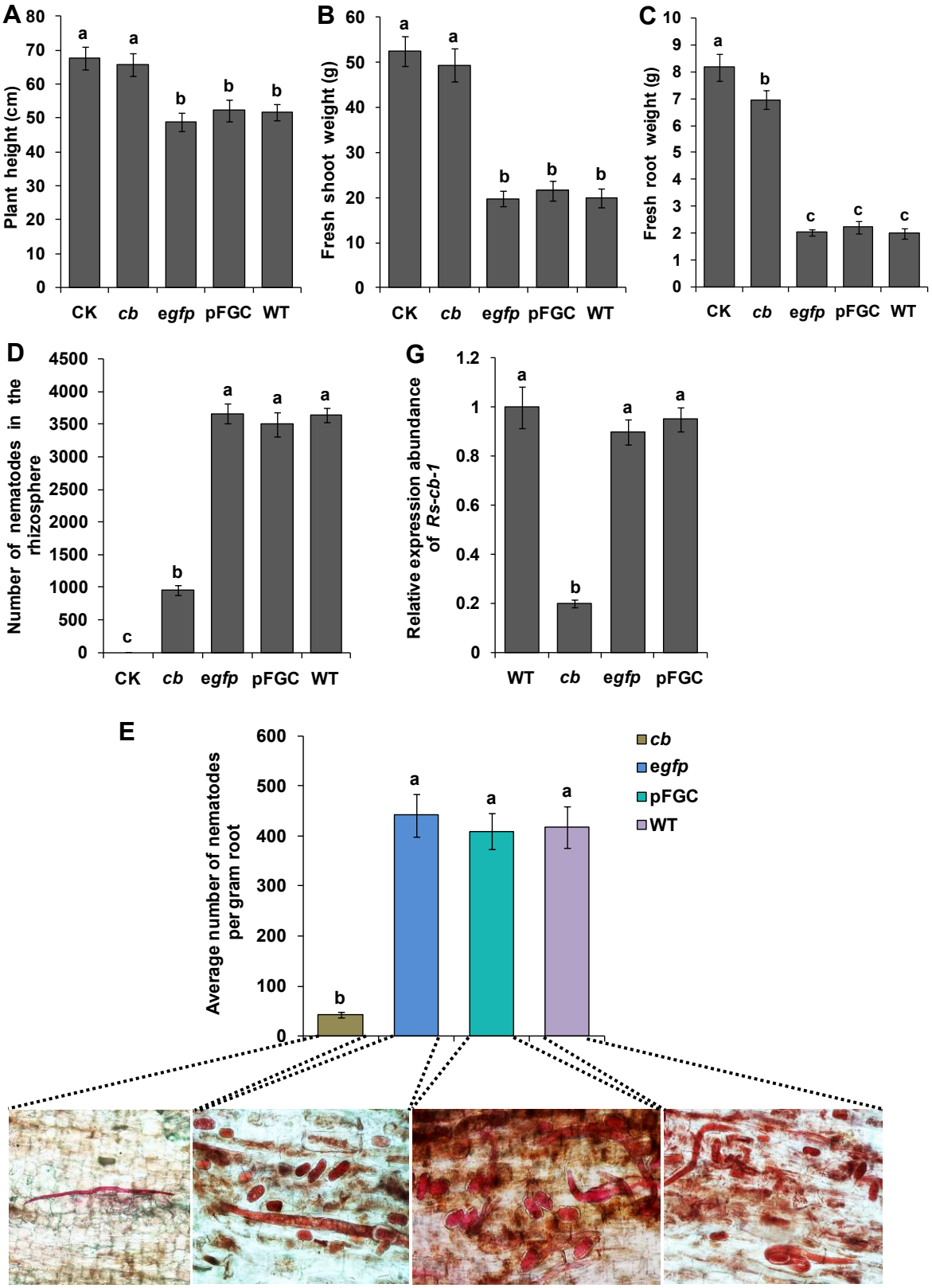


Figure S8


A
Plant height (cm)
B
Fresh shoot weight (g)
C
Fresh root weight (g)
CK
cb
egfp
pFGC
WT
D
Number of nematodes in the rhizosphere
G
Relative expression abundance of Rs-cb-1
E
Average number of nematodes per gram root
cb
egfp
pFGC
WT

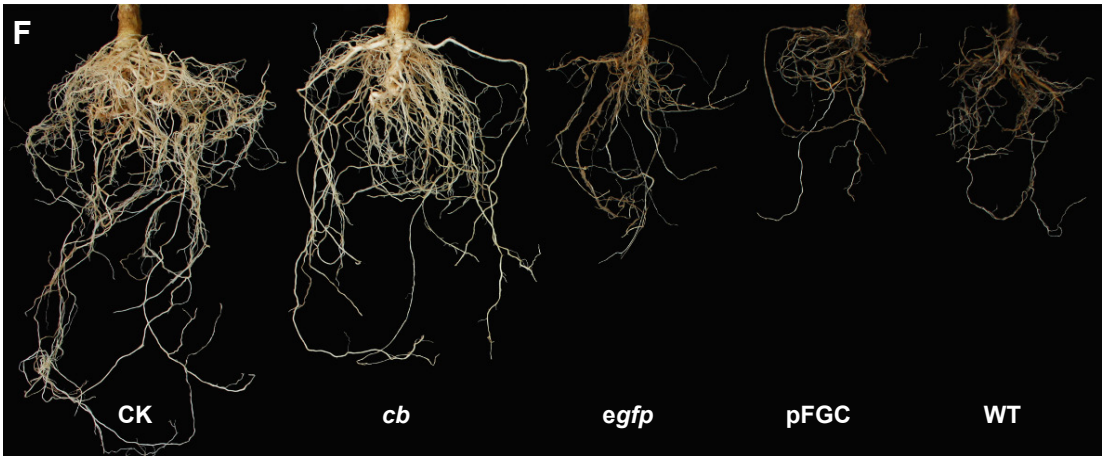
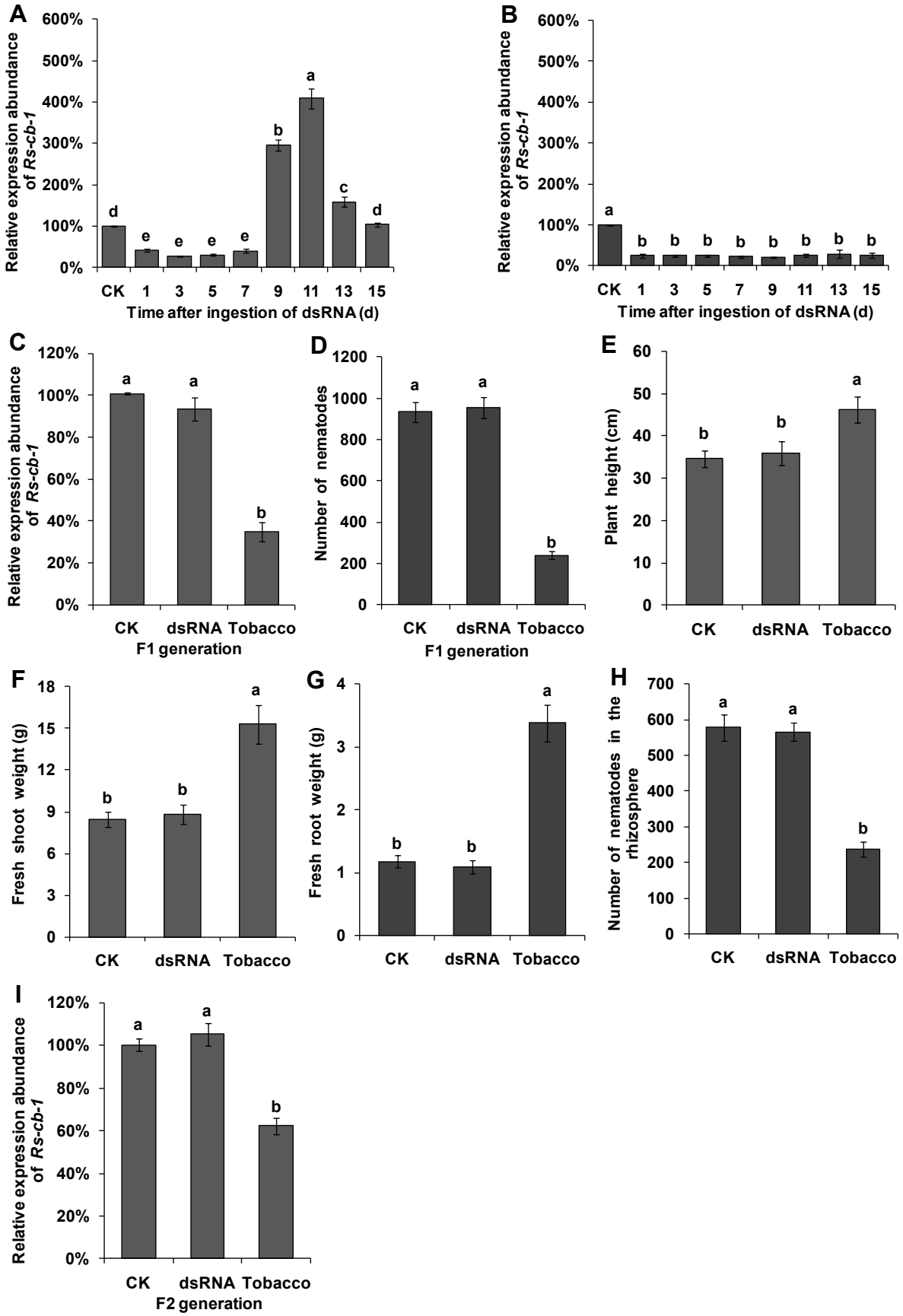

F
CK
cb
egfp
pFGC
WT

Figure S9


A
Relative expression abundance of Rs-cb-1
600%
500%
400%
300%
200%
100%
0%
d e e e e b a c d
CK 1 3 5 7 9 11 13 15
Time after ingestion of dsRNA (d)
B
Relative expression abundance of Rs-cb-1
600%
500%
400%
300%
200%
100%
0%
a b b b b b b b b
CK 1 3 5 7 9 11 13 15
Time after ingestion of dsRNA (d)
C
Relative expression abundance of Rs-cb-1
120%
100%
80%
60%
40%
20%
0%
a a b
CK dsRNA Tobacco
F1 generation
D
Number of nematodes
1200
1000
800
600
400
200
0
a a b
CK dsRNA Tobacco
F1 generation
E
Plant height (cm)
60
50
40
30
20
10
0
b b a
CK dsRNA Tobacco
F
Fresh shoot weight (g)
18
15
12
9
6
3
0
b b a
CK dsRNA Tobacco
G
Fresh root weight (g)
4
3
2
1
0
b b a
CK dsRNA Tobacco
H
Number of nematodes in the rhizosphere
700
600
500
400
300
200
100
0
a a b
CK dsRNA Tobacco
I
Relative expression abundance of Rs-cb-1
120%
100%
80%
60%
40%
20%
0%
a a b
CK dsRNA Tobacco
F2 generation