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# The RNA-binding domain of DCL3 is required for long-distance RNAi signaling

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[1] R L e P C , L e M , C A e , B 100101, C
[2] CA C e E B I , e C A e , B 100049, C
[3] Q A e M c M , J 250022, C

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**Abstract** RNA ( RNA)- RNA ( RNA e , RNA) RNA , DNA e RNA . H . I , RNA , *PDSi*, e *PDS* , DCL3 *PDS* RNA C- e DCL3, RNA- e RNA . M. RNA , *PDS* , DCL3 RNA e . C. e RNA- e DCL3 e RNA . O. DCL3 e 24- RNA .

**Keywords** RNA , DCL3, RBD, RNA , *Cucumber mosaic virus*

## INTRODUCTION

RNA ( RNA e , RNA) RNA , DNA e (B 2005; C 2009; G. 2018). RNA RNA ( RNA) e 21 24 ( ) .

(P G ) 21- 22- RNA , e RNA ( RNA) D DCL4 DCL2, RNA (C H 2004; G . 2005; M 2004).

RNA e ( G ). 24- RNA RNA DNA RNA-

✉ C : @ (J.-H. )

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DNA (RdDM) (J . 2002; M B 2005; 2008). L RNA DNA-RNA , P (C 2009; P . 2008). A P I -RNA- RNA , RDR2, RNA, DCL3 e 24- RNA (B . 2015; H . 2005; K . 2005; M . 2009; M B 2005; O . 2005; . 2019; . 2004; . 2007). A 24- RNA/AGO4/P e RNA DNA, e DRM2e - e (C J 2002; M . 2009). P I , RDR2 DCL3 e , G (D . 2009; K . 2008; P G 2019).

A RNA - (P . 1997; . 2007; B 1997). I RNA - (M . 2010; P G 2019). I RNA - (*IR*) *IR* (*-IR*) e P G R DM/ G e. *IR* e (G. . 2003). I *in cis* e G e. *-IR* *-IR* *IR*e RNA e e (D . 2011). F e *-IR* (., *-Pdsi*) e *-PDS*) *PDS*- (D . 2011; G. . 2003; L . 2019). M. e P DRD1, *-Pdsi* e *Pdsi* e *Pdsi*e RNA, e *PDS* (D . 2011). H P I , RDR2 DCL3 e 24- RNA e *-PDS* (D . 2011). I *dcl3* *nrpd1* ( NRPD1 eP I ) e *-PDS* (D . 2011).

24- RNA, DCL3 P I e RNA (RBD) e D /DCL D /DCL (B B 2014; D . 2013; J D 2009; N 2000; N 2014; R . 2000; . 2004).

H e DCL3 e *PDSi* C / P (CL ) e *-Pdsi* *-PDS* (F. 1A) (G. . 2003). C e *dcl3/PDSi* e DCL3 24- *-PDS* . H e *dcl3/PDSi* RBD C- e DCL3 *-PDS* . I e RNA e C- e DCL3 RNA . DCL3 e 24- RNA.

## MATERIALS AND METHODS

### Plant growth conditions and virus inoculation

*Arabidopsis* C -0 *PDSi* 2 (G. . 2003). *dcl3-5* M M .

*Arabidopsis* M 3% ( / ) 0.8% ( / ) . Ae 22 °C 16/8 . *N. benthamiana* 25 °C 16/8 . *PDS* 2 µ /L 17β . F e *PDS* M e M .

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F (CM -D ) *N. benthamiana* . - - *Arabidopsis* P 14 ( ).

## Cloning and plasmids

C E II C E M. ( , C ) e e . A 1.

BI121-6 -*DCL3* (NM 001161190.2, 4743 ) e *DCL3* C -0 B HI- BI121-M .

BI121-6 -*DCL3*$_{mRIII}$ BI121-6 -*DCL3*$_{\Delta RBD}$ e BI121-6 -*DCL3*, PCR B HI- BI121-M . F -*DCL3*-EGFP, -*DCL3*$_{mRIII}$-EGFP -*DCL3*$_{\Delta RBD}$ -*DCL3*, *DCL3*$_{mRIII}$ *DCL3*$_{\Delta RBD}$ I/B HI- -EGFP.

F e (G ) e (F1, F2, F3, F4 F5) e *DCL3* e BI121-6 -*DCL3* B HI/ I- GE -4 -2.

## Plant transformation

A (C . 1998) e e , *pDCL3*-6 -*DCL3*, *pDCL3*-6 -*DCL3*$_{mRIII}$ *pDCL3*-6 -*DCL3*$_{\Delta RBD}$ e *Agrobacterium* EHA105, - e *dcl3-5/PDSi*. P e M .

## RNA extraction and RNA gel blot analysis

F - e RNA , e RI (L - ) , e . 65 µ e RNA RNA e - RNA ( 1).

## qRT–PCR

e RNA, DNA ( ) DNA. R H II R . ( ). Q PCR e B -R CF 96 R - C Q BR PCR M M. ( ). *Arabidopsis* A 4, 33380 e 1.

## Purification of recombinant proteins and RNA binding activity detection

F e G - DCL3 e BL21 0.8 M -D-1- ( Ac ) L B 16 °C . G 4B (GE H - ) e G - e e .

F e (EM A), - RNA 0.3 M 40 e 4 RNA (P RNA 3 E B - K, 20,160) 50 . A e (D. . 2012). RNA (C N. A D M , 89,880). F (M ), (N , RED-NH 2 C ) e 30 M . (50 M HC H 7.4, 150 M N C, 10 M M C2 0.01% 20). F (100 M) . RNA e 5 . RNA (N , MO-K022) M N .115 (N , 2 °C, 15% 80% M ). MO.Ae A (.2.3)

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G P 6.0 e

A 2.

### Subcellular localization assays

e DCL3, DCL3 RIII DCL3 ΔRBD -*DCL3*-EGFP, -*DCL3*$_{mRIII}$-EGFP -*DCL3*$_{ΔRBD}$-EGFP *Agrobacterium* (EHA105). A 20 L e (LB 50 /L 25 /L e 10 M ME 20 M ) 28 °C 200 e 16 . *Agrobacterium* 10 M M C$_2$ (200 M ), 600 e 1.0. e 3 *N. benthamiana* e .

100 / L 49,6 -2- (DAPI) e 10 e e . C- e GFP DAPI B C P8, A P e (A ).

## RESULTS

### Reduced systemic *PDS* silencing phenotype in the *dcl3* mutant background

*dcl3-1/PDSi* -DNA *dcl3-1* ( ALK 005512) (D . 2011). I e *DCL3* *PDSi* , *dcl3-5* (F . 1B), *PDSi* (-2) (G . 2003). *dcl3-5/PDSi* (*dcl3/PDSi*) F1 - e F2 . H F3 e *PDSi* -2 7c (F . 1A). A , *dcl3/PDSi* *PDSi* (F . 1B), e *dcl3-1/PDSi* (D . 2011).

e *PDS* RNA . *PDS* RNA e *PDSi* *dcl3/PDSi* *PDSi* (*PDSi-un*) *dcl3/PDSi* (*dcl3/PDSi-un*) (F . 2A). e e *PDS* RNA c . A e *PDS* RNA e *PDSi* *dcl3/PDSi* e *PDSi-* *dcl3/PDSi-* (F . 2B). A- e 21- 24- RNA ( P P , P ) e *PDSi-* 21- P *dcl3/PDSi-* (F . 2C). A 24- RNA, A R 2, *dcl3/PDSi* R159 e - (F . 2C), e DCL3 DCL3 e 24- RNA - e *dcl3* e e .

### RNA binding activity of DCL3 is required for the induction of systemic silencing

A R DM , DCL3 e RNA 24- RNA ( . 2021; . 2022). RN III e - e -*PDS*. DCL3 PA RN III e RNA, DCL3 RBD (F . 3A). e *dcl3/PDSi* e *DCL3* , *DCL3*$_{mRIII}$, e RN III (F . 3A), , *DCL3*$_{ΔRBD}$, e C- e 89 RBD (F . 3A), DCL3 . A - *PDS*, *PDSi* (F . 1B 2A), e e *dcl3/PDSi/DCL3* *dcl3/PDSi/DCL3*$_{mRIII}$ - *dcl3/PDSi/DCL3*$_{ΔRBD}$ (F . 3B F . 2A). e *PDSi* *dcl3/PDSi* 7c (F . 2B), CL e

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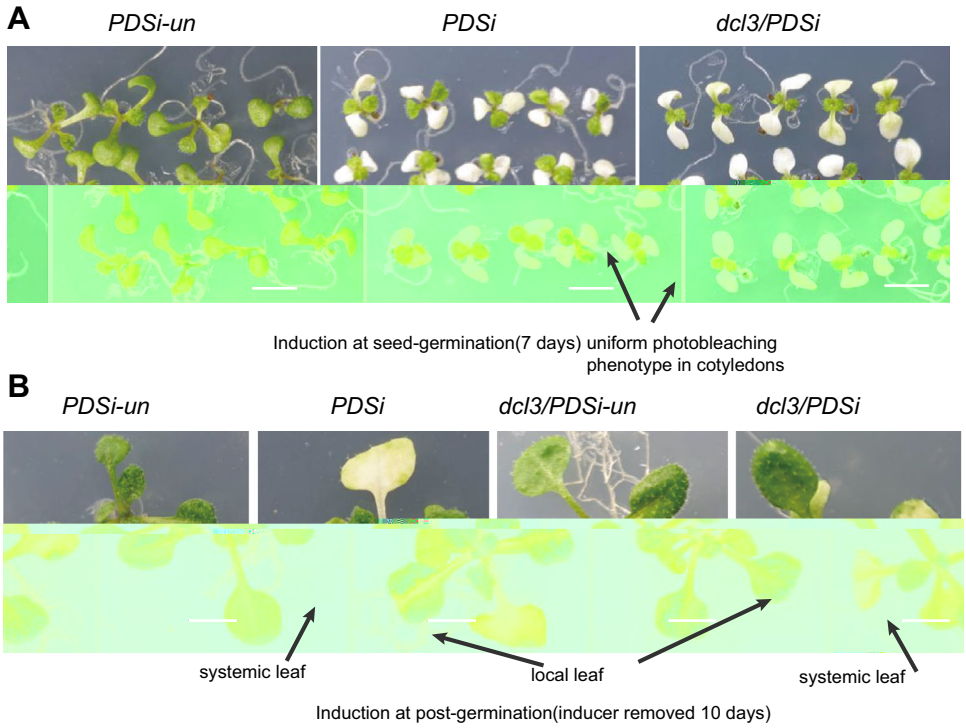


**Fig. 1** R . . . e. , *PDS* . . . *dcl3* . . . . **A** P . . e *PDS* . . . . . A . . e . . . . . *PDSi* . *dcl3/PDSi*. *PDSi-* : . . . . . B . = 0.5 . . **B** P . . e *PDS* . . . . . N *PDS* . . . . . *dcl3/PDSi*. P . . . . . 10 . e . . . . . B . = 0.5 .

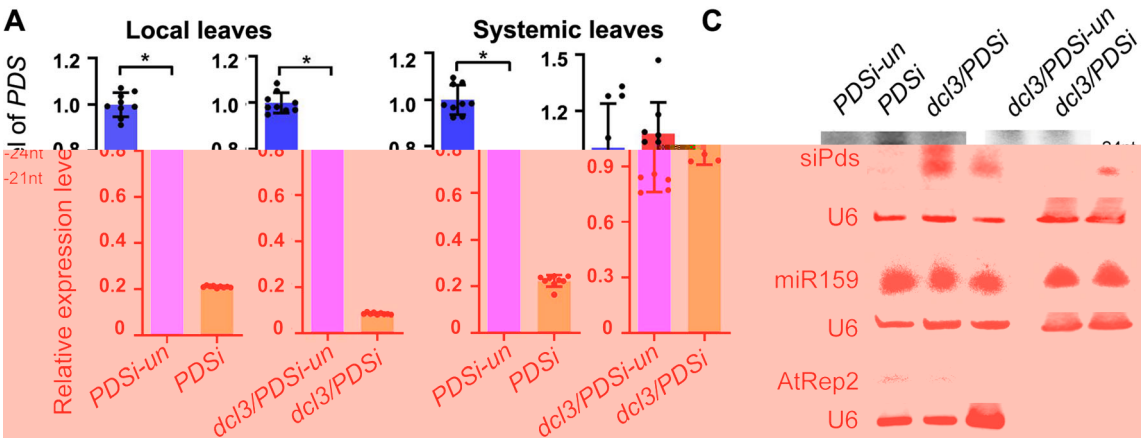


**Fig. 2** D . . . e *PDS* . RNA . . . e *PDS* e . . RNA . (R . ) . . . . . RNA . **A**, **B** . . e *PDS* . . . (**A**) . . . . (**B**) . . . e . . . . . ± D, . . . . . (*n* = 9, *t* . , *P* < 0.05). **C** D . . . . e . R . , A R 2 . . R159 . RNA . . . B . . . . . *PDS* RNA . . . . . e A R 2 . . R159 . . . 6 RNA . . . . . .

. . . . . RNA . . . . . e RNA . e DCL3 . . . e . . . . . e . . . -*PDS*.

. . . e DCL3, DCL3 $_{RIII}$ . DCL3$_{\Delta RBD}$ . . . . . *dcl3/PDSi/DCL3*, *dcl3/PDSi/DCL3$_{mRIII}$*, . *dcl3/PDSi/DCL3$_{\Delta RBD}$* . . . (F . 3). C . . . . . *PDSi-un* . . . e *PDS* . . . . . e *PDSi*, *dcl3/PDSi*, *dcl3/PDSi/DCL3*, *dcl3/PDSi/DCL3$_{mRIII}$* . *dcl3/PDSi/DCL3$_{\Delta RBD}$* . . . (F . 4A). C . . . . . *PDS* . . . . . *PDSi*, *dcl3/PDSi/DCL3*, . *dcl3/PDSi/DCL3$_{mRIII}$* . . . *dcl3/PDSi* . *dcl3/PDSi/*

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*DCL3$_{\Delta RBD}$* (F . 4B). N e
21-
24 *PDSi*, *dcl3/PDSi/DCL3*
*dcl3/PDSi/DCL3$_{\Delta RBD}$* (F . 4C), 21
*dcl3/PDSi* *dcl3/PDSi/DCL3$_{mRIII}$*. R159
e
(F . 4C). *dcl3/PDSi/DCL3$_{mRIII}$*
24 e
*-PDS* 24 RNA
e . N
21- 24 24 A 2
R1003 (F . 4C), e *dcl3/PDSi/*
*DCL3$_{\Delta RBD}$* e
e *-PDS* (F . 3B 4A).
21- 24

e *dcl3/PDSi/DCL3$_{\Delta RBD}$* (F . 4C),
e *PDS*
*dcl3/PDSi/DCL3$_{\Delta RBD}$* *PDSi-un* (F . 4B).
N , e
DCL3 $_{RIII}$ DCL3$_{\Delta RBD}$. DCL3, DCL3 $_{RIII}$ DCL3$_{\Delta}$-
$_{RBD}$ e EGFP *N.*
*benthamiana*.
(H . 2017), e EGFP
e DCL3-EGFP
(F . 4D). e RN III
RBD DCL3
(F . 4D).
RNA
e DCL3 RNA

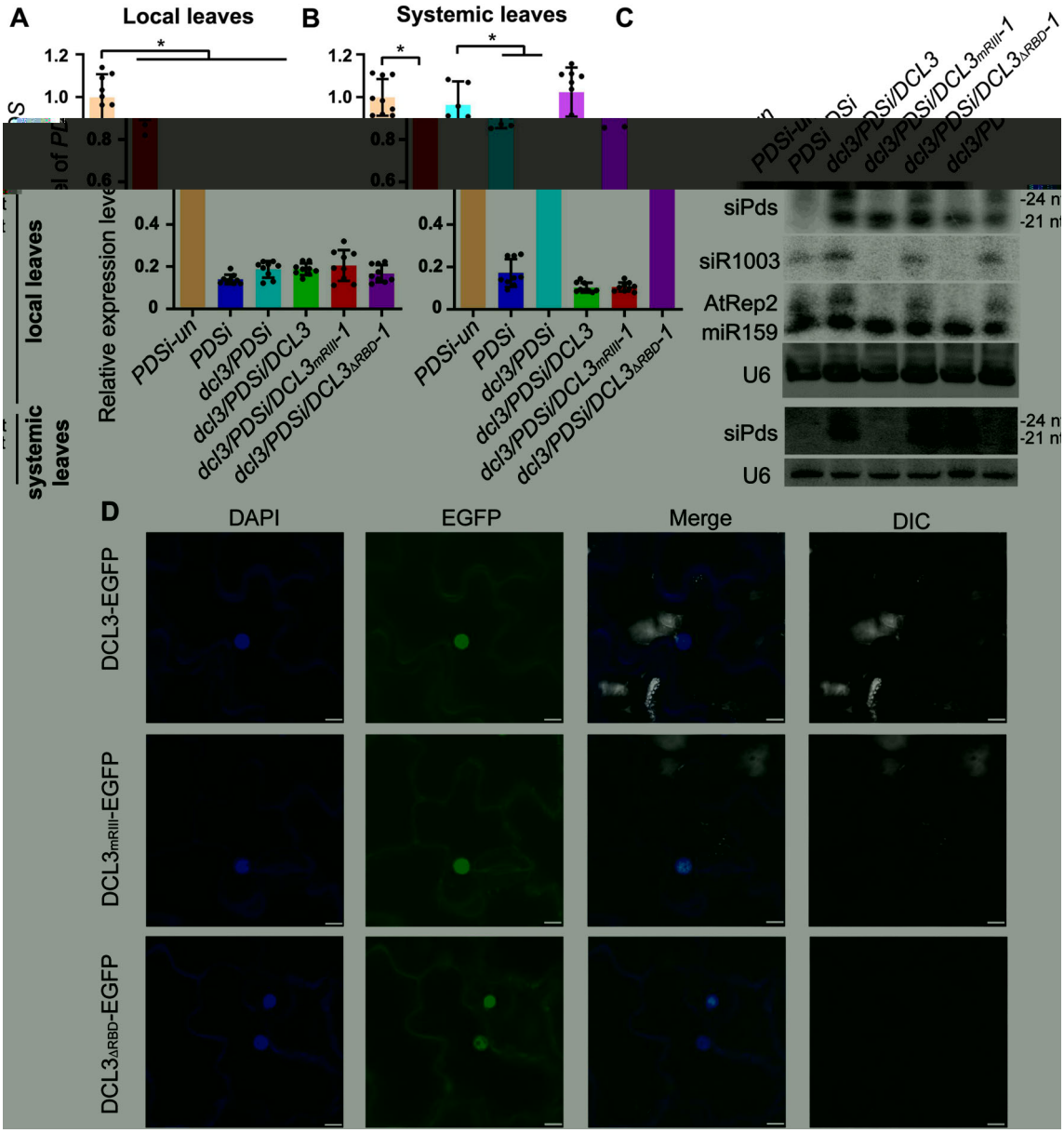


**Fig. 4** A e *PDS* DCL3 . **A**, **B** D e *PDS* RNA (A) (B) e ± D, e (*n* = 9, - ANO A, $P < 0.05$). **C** e *PDS*e RNA (R ), A R 2, R1003 R159 (RNA ). B - *PDS* RNA e A R 2 R159 RNA . H e A R 2 R159 . 6 RNA . **D** e DCL3, DCL3 RIII DCL3ΔRBD. P e e DAPI EGFP *N. benthamiana* EGFP e . O e e e e . B = 10 μ

## The C-terminal domain of DCL3 exhibits RNA binding activity

RNA e DCL3 EM A M . D e e DCL3, F1, F2, F3, F4 F5 (F . 5A), G e . F EM A , G G - 2 , CM e RNA (D. . 2012), G -2 e 21- ( ), 24- 55- - RNA (D. . 2012). EM A M F5, - RBD e e - RNA (F . 5B F 4A). E e F5, e e RNA (F . 5B F 4A). e e - RNA

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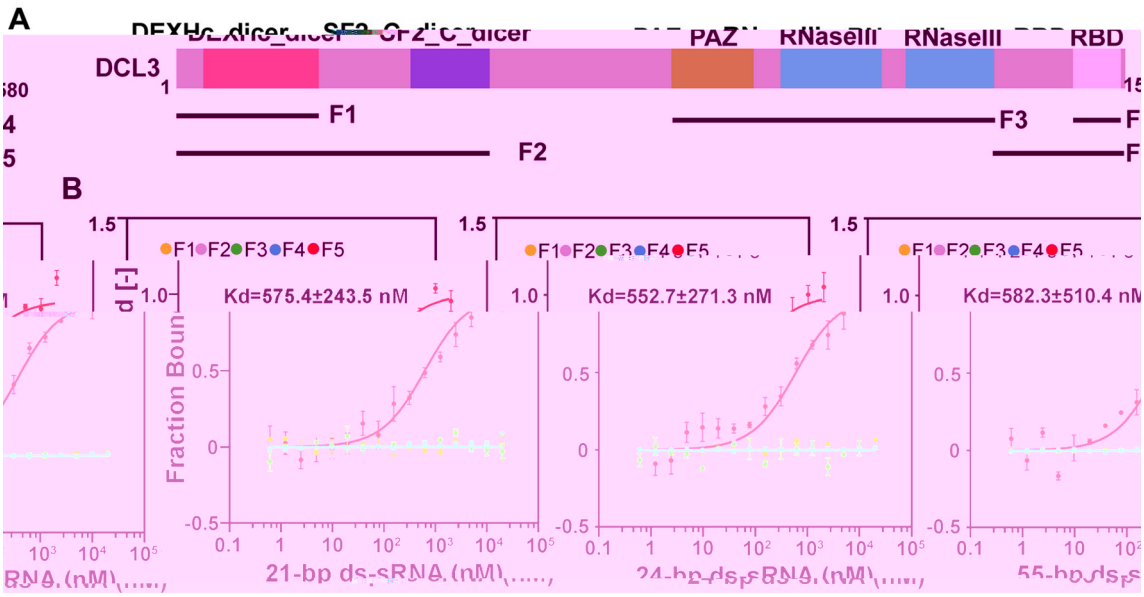


**Fig. 5** [illegible] RNA [illegible] eDCL3 [illegible] M [illegible]. **A** [illegible] G - [illegible] eDCL3 (F1, F2, F3, F4 [illegible] F5). **B** M [illegible] e [illegible] RNA [illegible] e [illegible] eDCL3. [illegible] G - [illegible] eDCL3 ([illegible] A) [illegible] 21-, 24- [illegible] 55- [illegible] RNA. 100 [illegible]

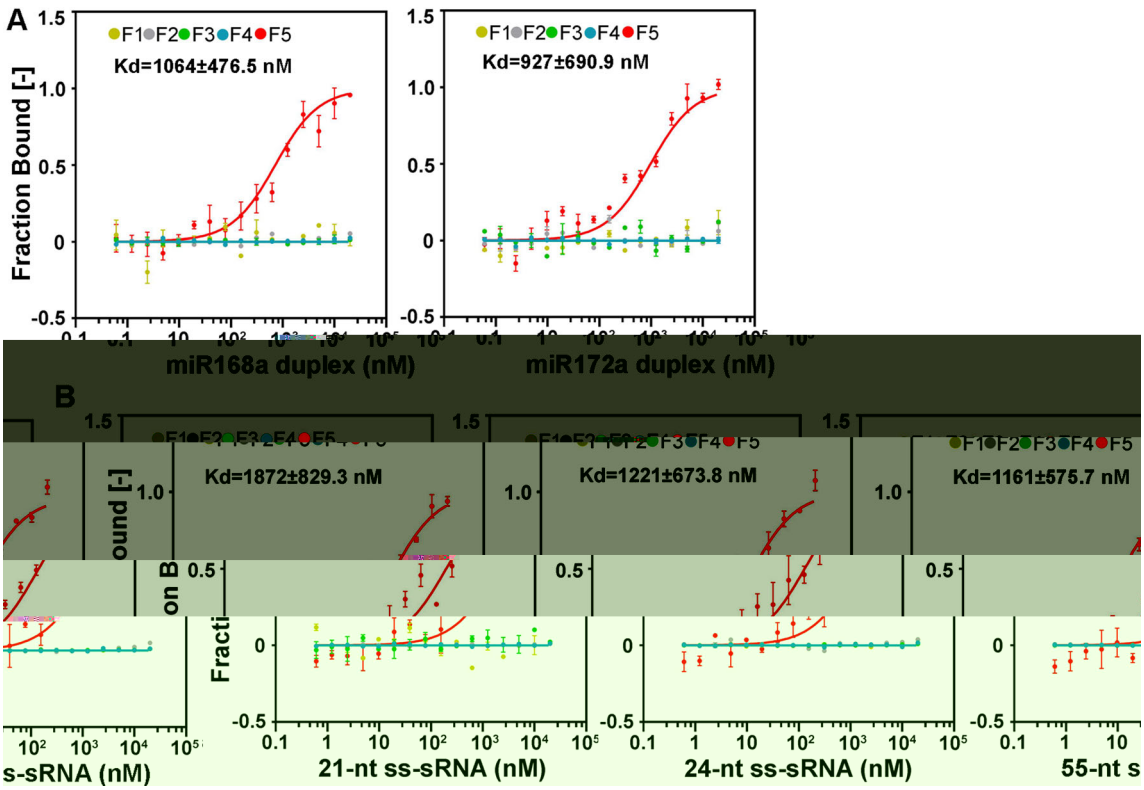


**Fig. 6** [illegible] RNA [illegible] RNA [illegible] RNA [illegible] eDCL3. **A** G - [illegible] F1, F2, F3, F4 [illegible] F5 [illegible] RNA [illegible] RNA [illegible]. **B** G - [illegible] F1, F2, F3, F4 [illegible] F5 [illegible] 21-, 24- [illegible] 55- [illegible] RNA. 100 [illegible]

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M , 575.4 ± 243.5 M e 21- RNA, 552.7 ± 271.3 M e 24- RNA 582.3 ± 510.4 M e 55- RNA (F . 5B).

2 , F5 e RNA . e RNA* , e 1064 ± 476.5 M e R168 927 ± 690.9 M e R172 (F . 6A F . 4B). H 2 , F5 e ( )- RNA e 21 ( = 1872 ± 829.3 M), 24 ( = 1221 ± 673.8 M), 55 ( = 1161 ± 575.7 M) (F . 6B F . 4C). O RBD RNA e DCL3, RBD RNA . DCL3 RNA e 21- RNA, 24- RNA, 55- RNA.

## RNA binding activity of DCL3 is required for systemic antiviral silencing

A DCL3 24- RNA RNA e DCL3 *dcl2/dcl4* (D 2007). e , RNA- e DCL3 e RNA e *Arabidopsis* CM , F . 7A, CM *PDSi*, *dcl3/PDSi/DCL3* *dcl3/PDSi/DCL3*$_{mRIII}$

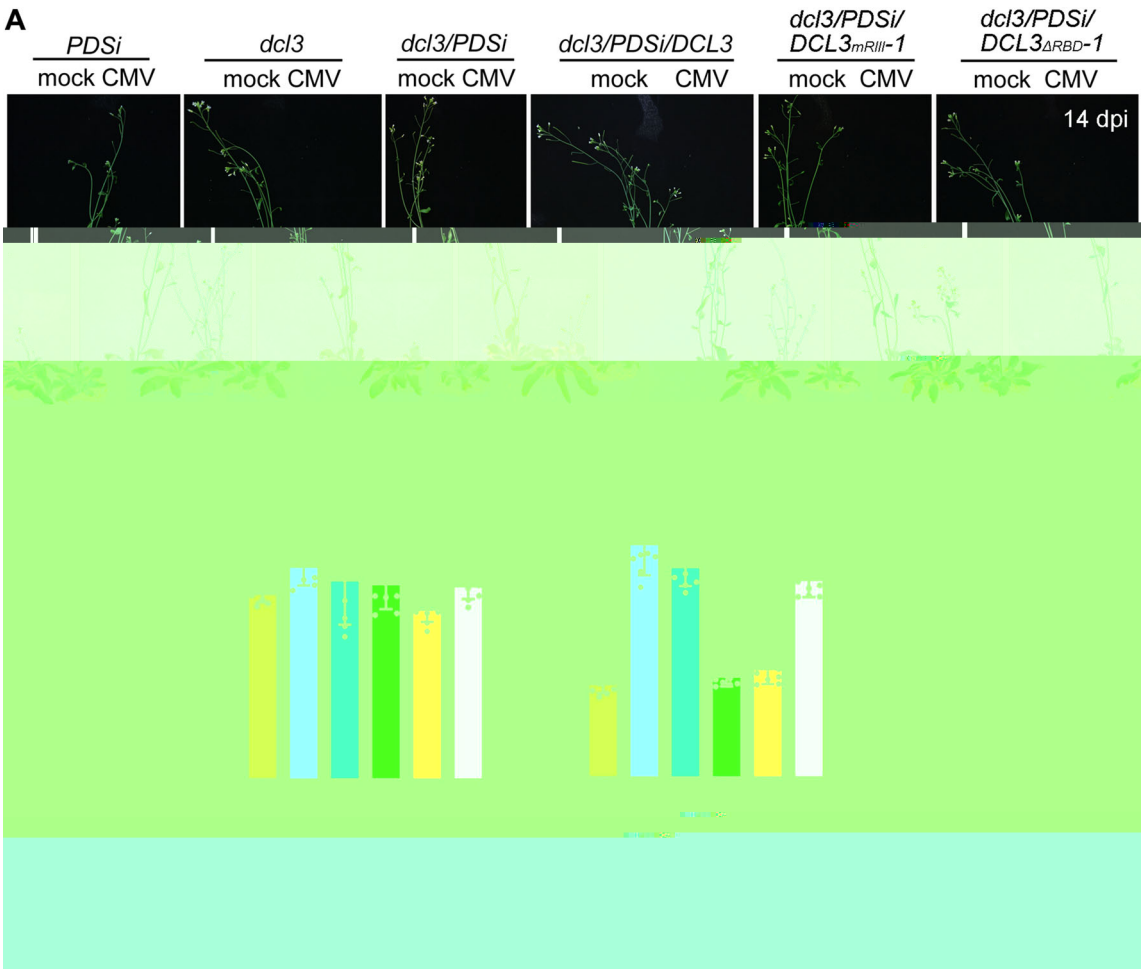


**Fig. 7** A e CM CM RNA . **A** CM *PDSi*, *dcl3*, *dcl3/PDSi*, *dcl3/PDSi/DCL3*, *dcl3/PDSi/DCL3*$_{mRIII}$ *dcl3/PDSi/DCl3*$_{\Delta RBD}$. P 14 B = 5 . **B**, **C** D e CM RNA R PCR. RNA , CM , (**B**) (**C**) 11 ± D, (*n* = 9, - ANO A, $P < 0.05$)

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CM *dcl3*, *dcl3/PDSi* *dcl3/PDSi/DCL3*$_{\Delta RBD}$ e (F . 7A). C R PCR RNA e CM *dcl3*, *dcl3/PDSi* *dcl3/PDSi/DCL3*$_{\Delta RBD}$ e *PDSi*, *dcl3/PDSi/DCL3* *dcl3/PDSi/DCL3*$_{mRIII}$ e RNA (F . 7B C). DCL3 -CM RNA- e RNA e .

## DISCUSSION

C / P -*Pdsi* RNA -*PDS* e RNA e (G. . 2003). e *PDS* RNA (D .54 e2.01 e1.8602.

P D RBD e e D (B B 2014; D 2013). D DCL3 e RBD e DCL3 e DCL3 e DCL3 ( :// / ), DCL3 RNA e RNA e 24 RNA e DNA . H DCL3 e DCL3 . DCL3 e DCL3 RNA e

**Supplementary Information** :// /10.1007/ 42994-023-00124-6.

**Acknowledgements** M M e *dcl3-5* L D e *dcl3-5* *PDSi* .

**Author contributions** JL, H G JH JL, B , H CLL e . JL, JH , H G . JL, H G JH .

**Funding** N N F e C (32020103003) P C C C P (C N . 2022DB014).

**Data availability** A .

**Declarations**

**Conflict of interest** .

**Open Access** C A 4.0 L , e ( ) C , e C Ie C :// / /4.0/.

## References

B , B P (2014) F e RNA- . RNA B 11:1226 1232. :// /10.4161/15476286.2014.972856

B D (2005) RNA . B 30:290 293. :// /10.1016/ .2005.04.012

B (2015) eP I RDR2e e24 RNA DNA *Arabidopsis*. E 4: 09591. :// /10.7554/ L .09591

C , J E (2002) R e *Arabidopsis* DRM DNA . C B 12:1138 1144. :// /10.1016/ 0960-9822(02)00925-9

C MA, H GJ (2004) RN III e . N M B 11:214 218. :// /10.1038/ 729

C JK (2009) RNAe DNA . C L 52:331 343. :// /10.1007/ 11427-009-0052-1

C , B AF (1998) F : e *Agrobacterium*- e *Arabidopsis thaliana*. P J 16:735 743. :// /10.1046/ .1365-313 .1998.00343.

D L, K , B E, J J, N , M AJ, M M (2009) A e 24 RNA e DNA . EMBO J 28:48 57

D O (2007) A RNA . C 130:413 426

D L (2011) DRD1-P e e e . P J 68:633 645. :// /10.1111/ .1365-313 .2011.04714.

D M (2013) RNA e D e RNA 19:1238 1252. :// /10.1261/ .039255.113

D. CG (2012) e *Arabidopsis* ARGONA E1- RNA DNA e *Cucumber mosaic virus* 2 . P C 24:259 274. :// /10.1105/ .111.092718

G , M AC, B DP, H (2005) P e e *Arabidopsis* DICER- e DCL4 RNA . C B 15:1494 1500. :// /10.1016/ .2005.07.024

G. H, J, Q C NH (2003) A RNA . P J 34:383 392. :// /10.1046/ .1365-313 .2003.01723.

G. B, , L , G -O A, D (2018) e e e RNA e RNA . P P 176:1587 1597. :// /10.1104/ .17.01370

H A, M, D , B DJ (2005) RNA I e DNA 308:118 120. :// /10.1126/ .1106910

H CM, C IR, K, A , N, M AH (2017) BA4: *Arabidopsis subcellular* . N A R 45:1064 1074. :// /10.1093/ / 1041

J J, L A, C , J JN (2002) C e C N G DNA KR P ONI E H3

© A 2023, 2023

. N 416:556 560. :// /10.1038/ 731
M, D JA (2009) A e RNA . N 457:405 412. :// /10.1038/ 07755
, A. e , J E, M MF, M M, M AJ (2005) A NF2- e e DNA . EMBO R 6:649 655. :// /10.1038/ .7400446
(2008) A - e RNAe DNA . N G 40:670 675. :// /10.1038/ .119
J, G. H (2019) IBM1e H3K9c e e e e . J G G 46:149 153. :// /10.1016/ . 2019.02.006
A, M A, E M, M A, C , O (2013) R . N G 45:1029 1039. :// /10.1038/ .2703