New Phytologist
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Article title: **Endogenous activated siRNAs in virus-infected *Brassicaceae* crops show a common host gene silencing pattern affecting photosynthesis and stress-response.**
Authors: Paola Leonetti, Aysan Ghasemzadeh, Arianna Consiglio, Torsten Gursinsky, Sven-Erik Behrens, Vitantonio Pantaleo.
Article acceptance date: 4th September 2020
The following Supporting Information is available for this article:

Fig. S1. Schematic representation of the machine learning structure for image analyses and tissues selection. (**a**) Input leaves images from either mock-treated or CaMV-infected turnip and oilseed rape were submitted to a machine learning process with the architecture of DeepLab v3+ CNN based classifier in order to classify the leaves in symptomatic or symptomless. (**b**) Accuracy of the classifier, determined over the validation set.

(a)

Neuronal Network Architecture of the DeepLab v3+ CNN based classifier
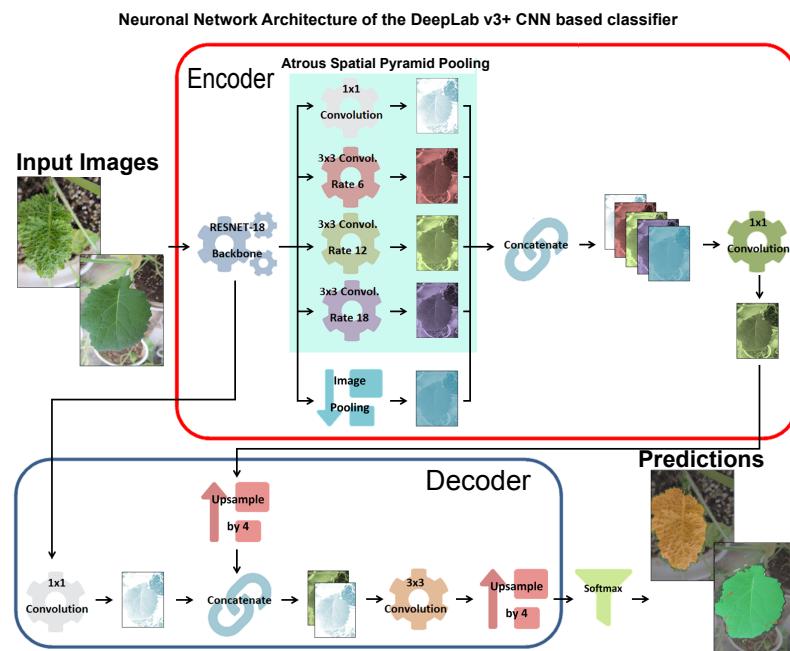
(b)

Ground truth classes	Accuracy
Background	0.94928
Symptomless leaves	0.93291
Symptomatic leaves	0.97427

Fig. S2. Size distribution profile of unique and redundant CaMV-derived siRNAs (vsiRNAs), in Turnip and Oilseed Rape.

CaMV-derived vsiRNAs in *Brassica napus*

CaMV-derived vsiRNAs in *Brassica napus*
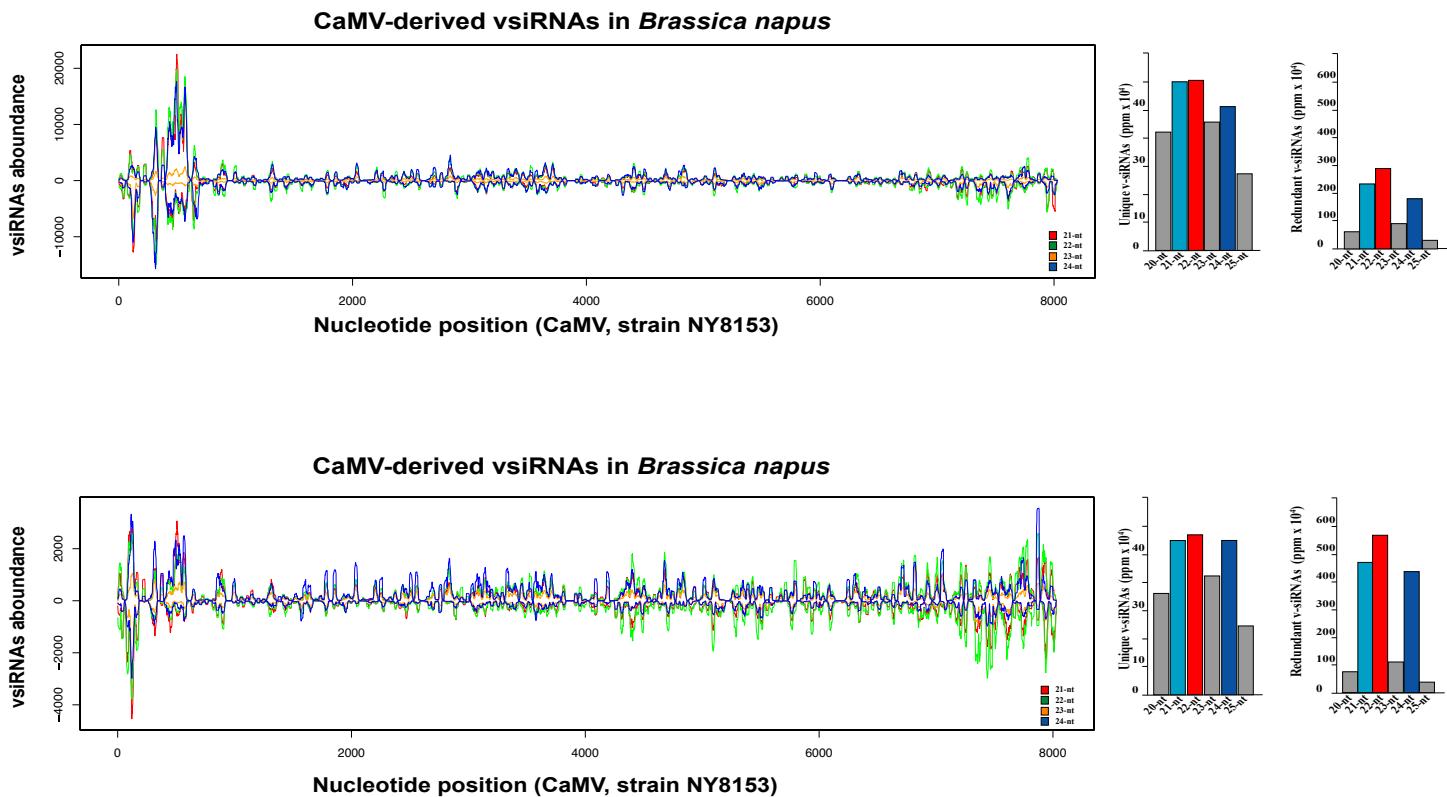
Fig. S3. Origin of sRNAs in *Arabidopsis thaliana*, Turnip and Oilseed Rape infected by CaMV. (**a**) Length distribution and abundance (reads per million $\times 10^3$) of the unique sRNAs derived from ribosomal (r)RNAs. (**b**) Distribution and abundance (reads per million $\times 10^4$) of the unique sRNAs derived from transposons. M=mock-inoculated, I=CaMV-infected plants.
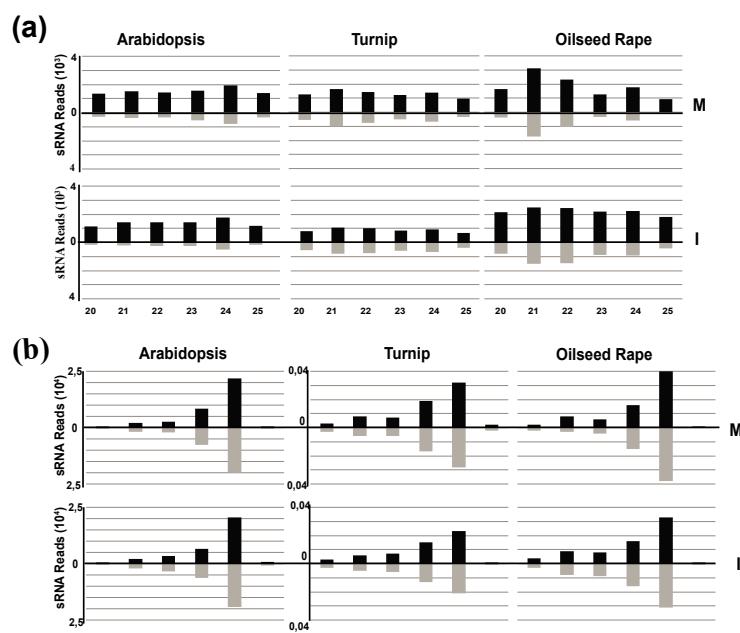
Fig. S4. **PCR amplicons for T7 in vitro transcripts used in cleavage assays.** Amplified products were eluted from the gel with Kit Promega in 40 µl water following the manufacturer instructions. 5 µl of DNA fragment was resolved in 1.2% agarose in order to verify the expected size. 5 µl were sequenced using the reverse oligonucleotide (denoted as "R" in **Table S4**). Amplicon T7 RCA of 875 bp (lane 1), T7 HSP70-1 of 562 bp (lane 2), T7 LHCB1.3 of 500 bp (lane 3). In the gel the marker is a 100bp Ladder (L, New England Biolabs). Oligonucleotides sequences are in **Table S4**.
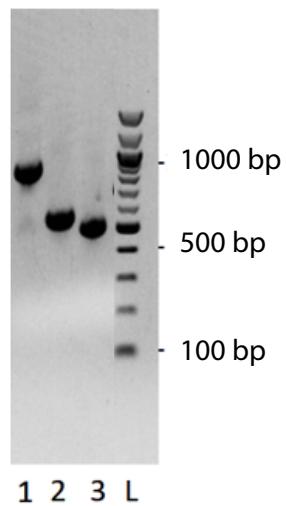
Fig. S5. **Alignments of the Sanger-sequenced amplicons (RCA, HSP70-1, LHCB 1.3) obtained from Turnip with the orthologues in Oilseed Rape.** Highlighted in violet are the oligonucleotides used to generate the DNA amplicons of RCA, HSP70-1, LHCB 1.3 and reported in **Table S4**. In red and in blue are the targets of siRNA effector #1 and effector #2, respectively, and highlighted in yellow the putative cleavage site. The length of 5'-products of cleaved transcript are indicated in bold and are reported above each target site. Default parameters were used for CLUSTAL Omega (1.2.4) aligner.

RCA

CLUSTAL Omega (1.2.4) multiple sequence alignment
https://plants.ensembl.org/ CDX93240= BnaC04g46560D-1

```
Bra017055.1                              GGGAAAGAAAGTTGTAACCGCGTCGAGATTCGCACA        137
BnaC04g46560D-1        T7p(22)GGGAAAGAAAGTTGTAACCGCGTCGAGATTCGCACA        420
                                         ***********************************

Bra017055.1     GAACAACAAGAAGAGCAACGGATCATTCAAGGTGGTTGCCGTGAAAGAAGACAAACAAAC        197
BnaC04g46560D-1 GAACAACAAGAAGAGCAACGGATCATTCAAGGTGGTTGCCGTGAAAGAAGACAAACAAAC        480
                ************************************************************

Bra017055.1     CGATGGAGACAGATGGAGGGGACTTGCCTACGACATGTCTGATGATCAACAAGACATCAC        257
BnaC04g46560D-1 CGATGGAGACAGATGGAGGGGACTTGCCTACGACACATCTGATGACCAACAAGACATCAC        540
                ***********************************  ******** **************

Bra017055.1     CAGAGGCAAAGGTATGGTTGACTCAGTCTTCCAAGCTCCTATGGGAACCGGAACTCACAA        317
BnaC04g46560D-1 CAGAGGCAAAGGTATGGTTGACTCAGTCTTCCAAGCTCCTATGGGAACCGGAACTCACAA        600
                ************************************************************
                      Cleavage RCA_1: 261            Cleavage RCA_2: 293
Bra017055.1     CGCCGTCCTTAGCTCCTATGAGTACATCAGCCAAGGTCTTAAGCAGTACAACTTGGACAA        377
BnaC04g46560D-1 CGCCGTCCTTAGCTCCTATGAGTACCTCAGCCAAGGTCTTAAGCAGTACAACTTGGACAA        660
                *********************** * ******* **************************

Bra017055.1     GATGATGGATGGGCTTTACATTGCCCCTGCTTTCATGGACAAGCTTGTTGTTCACATCAC        437
BnaC04g46560D-1 CATGATGGATGGGCTTTACATTGCCCCTGCTTTCATGGACAAGCTTGTTGTTCACATCAC        720
                 ***********************************************************

Bra017055.1     CAAGAACTTCTTGACTTTGCCTAACATCAAGGTTCCACTTATTTTGGGTGTTTGGGGAGG        497
BnaC04g46560D-1 CAAGAACTTCTTGACTTTGCCTAACATCAAGGTTCCACTTATTTTGGGTGTTTGGGGAGG        780
                ************************************************************

Bra017055.1     CAAAGGTCAAGGTAAATCCTTCCAGTGTGAGCTTGTCATGGCCAAGATGGGCATCAACCC        557
BnaC04g46560D-1 CAAAGGTCAAGGTAAATCCTTCCAGTGTGAGCTTGTCATGGCCAAGATGGGCATCAACCC        840
                ************************************************************

Bra017055.1     AATCATGATGAGTGCTGGAGAGCTTGAGAGCGGGAACGCAGGAGAGCCAGCCAAGCTTAT        617
BnaC04g46560D-1 AATCATGATGAGTGCTGGAGAGCTTGAGAGCGGGAACGCAGGAGAGCCAGCCAAGCTTAT        900
                ************************************************************

Bra017055.1     CCGTCAAAGGTACCGCGAGGCAGCAGACCTGATCAAGAAAGGAAAGATGTGTTGTCTCTT        677
BnaC04g46560D-1 CCGTCAAAGGTACCGTGAGGCAGCAGACCTGATCAAGAAAGGAAAGATGTGTTGTCTCTT        960
                *************** ********************************************

Bra017055.1     CATCAACGATCTCGACGCTGGTGCTGGTCGTATGGGTGGTACCACACAGTACACTGTCAA        737
BnaC04g46560D-1 CATCAACGATCTCGACGCTGGTGCTGGTCGTATGGGTGGTACTACACAATACACTGTCAA        1020
                ****************************************** ***** **********

Bra017055.1     CAACCAGATGGTTAACGCAACGCTCATGAACATTGCTGATAACCCCACCAACGTCCAGCT        797
BnaC04g46560D-1 CAACCAGATGGTTAACGCAACGCTCATGAACATTGCTGATAACCCCACCAACGTCCAGCT        1080
                ************************************************************

Bra017055.1     CCCAGGAATGTACAACAAGGAAGACAACGCACGTGTCCCCATCATCGTCACCGGTAACGA        857
BnaC04g46560D-1 CCCAGGAATGTACAACAAGGAAGACAACGCACGTGTCCCCATCATCGTCACCGGTAACGA        1140
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Bra017055.1      TTTCTCCACCCTCTACGCTCCTCTCATCCGTGATGGACGTATGGAGAAGTTCTACTGGGC    917
BnaC04g46560D-1  TTTCTCCACTCTCTACGCTCCTCTCATCCGTGATGGACGTATGGAGAAGTTCTACTGGGC    1200
                 ********* **************************************************


Bra017055.1      CCCGACCCGTGAGGACCGTATCGGTGTCTGCAAGGGT         954
BnaC04g46560D-1  CCCGACCCGTGAGGATCGTATCGGTGTCTGCAAGGGT         1237
                 *************** *********************
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HSP70-1

CLUSTAL Omega (1.2.4) multiple sequence alignment
https://plants.ensembl.org/ CDY16797= BnaA09g05850D-1

```
Bra035909.1             GGAATCCCTCCAGCTCCACGTGGTGTCCCCCAAATCACTGTCTGCTTCGACATTGACGCC    1239
BnaA09g05850D-1  T7p(22)GGAATCCCTCCAGCTCCACGTGGTGTCCCTCAGATCACTGTCTGCTTCGACATTGACGCC    384
                        **************************** **  *************************

Bra035909.1      AACGGTATCCTCAACGTCTCTGCCGAGGACAAGACCCACGGACAGAAGAACAAGATCACC    1299
BnaA09g05850D-1  AACGGTATCCTCAACGTCTCTGCTGAGGACAAGACCCACGGACAGAAGAACAAGATCACC    444
                 *********************** ************************************
Cleavage HSP70.1_1:143
Bra035909.1      ATCACCAACGACAAGGGTCGTTTGTCCAAGGACGATATCGAGAAGATGGTTCAAGAGGCT    1359
BnaA09g05850D-1  ATCACCAACGACAAGGGTCGTTTGTCCAAGGACGATATCGAGAAGATGGTTCAAGAGGCT    504
                 ****  ******** *****************************************

Bra035909.1      GAGAAGTACAAGTCTGAGGATGAGGAGCACAAGAAGAAGGTGGAAGCCAAGAACGCTCTC    1419
BnaA09g05850D-1  GAGAAGTACAAGTCTGAGGATGAGGAGCACAAGAAGAAGGTGGAAGCCAAGAACGCGCTC    564
                 ****************************************************** ***

Bra035909.1      GAGAACTACGCGTACAACATGAGGAACACCATCCAGGACGACAAGATTGGTGAGAAGCTA    1479
BnaA09g05850D-1  GAGAACTACGCCTACAACATGAGGAACACCATCCAGGACGACAAGATTGGTGAGAAGCAT    624
                 *********** **************************************************

Bra035909.1      CCGGCTGCGGACAAGAAGAAGATCGAGGACTCTATTGAGCAGGCGATTCAGTGGCTGGAG    1539
BnaA09g05850D-1  CCGGCTGCAGACAAGAAGAAGATCGAGGAGTCTATTGAGCAGGCGATTCAGTGGCTGGAG    684
                 ********  ****************** ******************************

Bra035909.1      AACAATCAGCTGGGTGAGGCTGATGAGTTTGAAGACAAGATGAAGGAGTTGGAGAGCATC    1599
BnaA09g05850D-1  AATAATCAGTTGGGTGAGGCTGATGAGTTCGAAGACAAGATGAAGGAGTTGGAGAGCATC    744
                 **  ** *** ****************** *****************************
                     Cleavage HSP70.1_2: 467
Bra035909.1      TGCAACCCAATCATTGCCAAGATGTACCAAGGAGCTGGTGGTGAAGCCGCAGGGATGGAC    1659
BnaA09g05850D-1   TGCAACCCAATCATTGCCAAGATGTACCAAGGAGCTGGTGGTGAAGCCGCTGGGATGGAC    804
                  ******************** *************** *********

Bra035909.1      GATGATGATGCTCCTCCTGCTTCAGGCGGTGCTGGCCCTAAGATCGAAGAGGTCGACTAA    1719
BnaA09g05850D-1   GATGATGATGCTCCTCCTGCCTCTGGCGGTGCTGGCCCTAAGATCGAAGAGGTCGACTAA    864
                  ****************** ** ************
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LHCB1.3

CLUSTAL Omega (1.2.4) multiple sequence alignment
https://plants.ensembl.org/ CDY35271= BnaC03g59520D-1

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Bra010807.1                GGCCGTGTGACAATGGGGAAGACCGTAGCCAAGCCAAAGG    130
BnaC03g59520D-1  T7p(22)GGCCGTGTGACAATGAGGAAGACCGTAGCCAAGCCAAAGG    240
                        ************** **********************

Bra010807.1      GCCCATCAGGCAGCCCATGGTACGGGTCCGAAAGAGTCAAGTACTTGGGCCCATTCTCCG    190
BnaC03g59520D-1  GCCCATCAGGCAGCCCATGGTACGGATCCGAGAGAGTCAAGTACTTGGGCCCATTCTCCG    300
                 ************************* ****  ***************************
                      Cleavage LHCB1.3_1: 159
Bra010807.1      GCGAGCCACCGAGCTACCTTACCGGAGAGTTCCCAGGAGACTACGGATGGGACACCGCAG    250
BnaC03g59520D-1  GCGAGCCACCGAGCTACCTTACCGGAGAGTTCCCAGGAGACTACGGATGGGACACCGCAG    360
                 ************************************************************
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Bra010807.1      GTCTCTCAGCCGATCCCGAGACGTTCGCAAGGAACCGTGAGCTAGAAGTTATCCACTGCA        310
BnaC03g59520D-1  GTCTCTCAGCCGATCCCGAGACGTTCGCGAGGAACCGTGAGCTAGAAGTTATCCACTGCA        420
                 ****************************  ******************************


Bra010807.1      GGTGGGCCATGCTCGGAGCCCTAGGCTGCGTCTTCCCTGAGTTGTTGGCCAGGAACGGAG        370
BnaC03g59520D-1  GGTGGGCCATGCTCGGAGCCCTAGGCTGCGTCTTCCCGGAGCTGTTGGCCAGGAACGGAG        480
                 *************************************  *** *****************
                                                       Cleavage LHCB1.3_1: 354
Bra010807.1      TCAAGTTCGGAGAGGCGGTTTGGTTCAAGGCTGGTTCACAGATCTTCAGCGAAGGAGGGC        430
BnaC03g59520D-1  TCAAGTTCGGAGAGGCGGTTTGGTTCAAGGCCGGTTCGCAGATCTTCAGCGAAGGAGGAC        540
                 *******************************  ***** ******************* *


Bra010807.1      TTGACTACTTGGGAAACCCTAGCTTGGTTCACGCTCAGAGCATCTTGGCTATTTGGGCTA        490
BnaC03g59520D-1  TTGATTACTTGGGAAACCCTAGCTTGGTTCACGCTCAGAGCATCTTGGCTATTTGGGCTA        600
                 ****  ******************************************************


Bra010807.1      CTCAAGTGATCTTGATGGGAGCCGTTGAAGGCTACAGAGTCGCAGGAGATGGGCCGTTGG        550
BnaC03g59520D-1  CTCAAGTGATCTTGATGGGAGCCGTTGAAGGTTACAGAGTCGCAGGAGATGGGCCGTTGC        660
                 *******************************  ****************************


Bra010807.1      GAGAGGCCGAGGACTTGC    568
BnaC03g59520D-1  GAGAGGCCGAGGACTTGC    678
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```

(a) Turnip RCA (*Bra017055.1*)

read length 21-nt
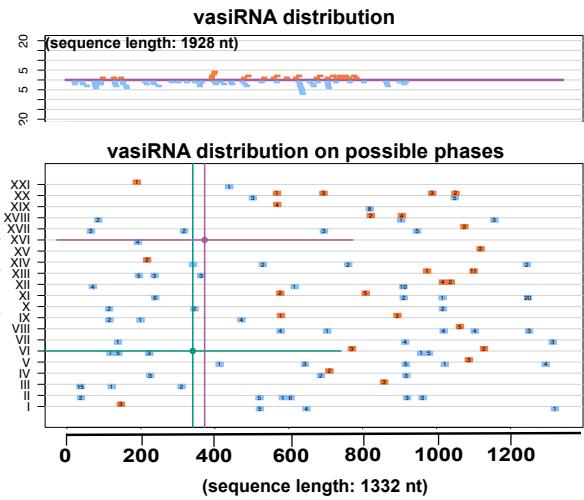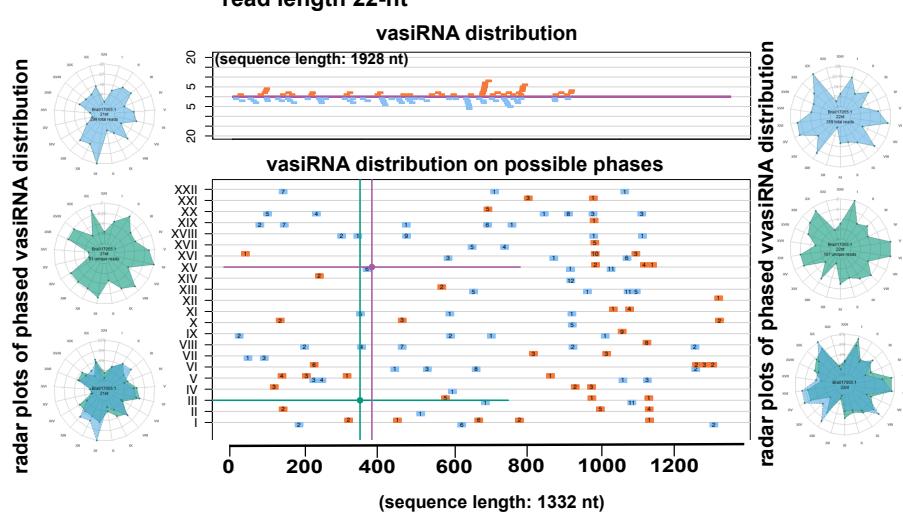
read length 22-nt

(b) Oilseed Rape (*BnaC04g46560D-1*)

read length 21-nt
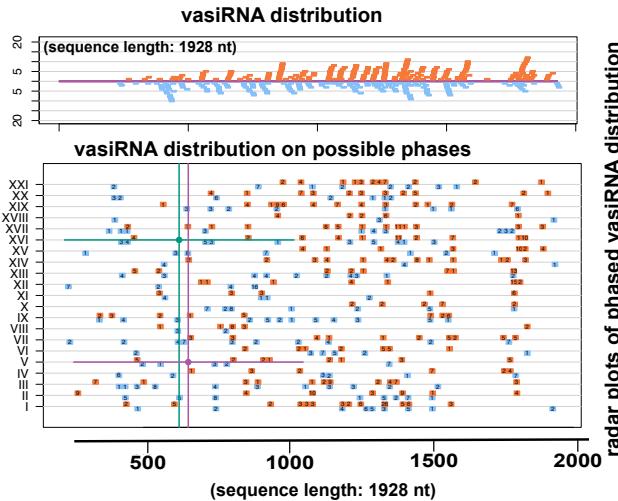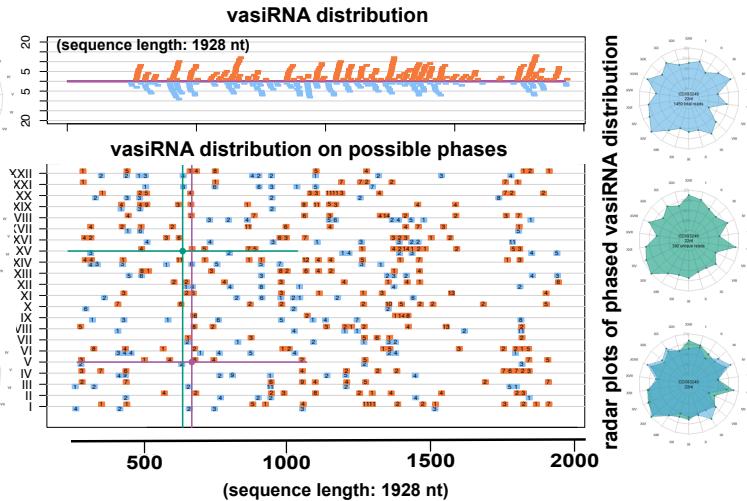
read length 22-nt

(c) Turnip HSP70-1 (*Bra035909.1*)

read length 21-nt
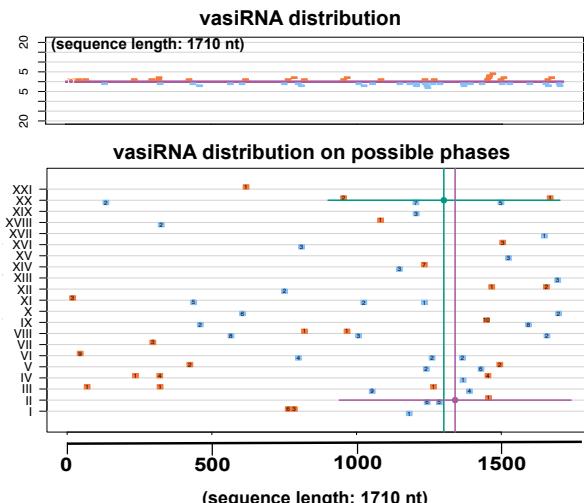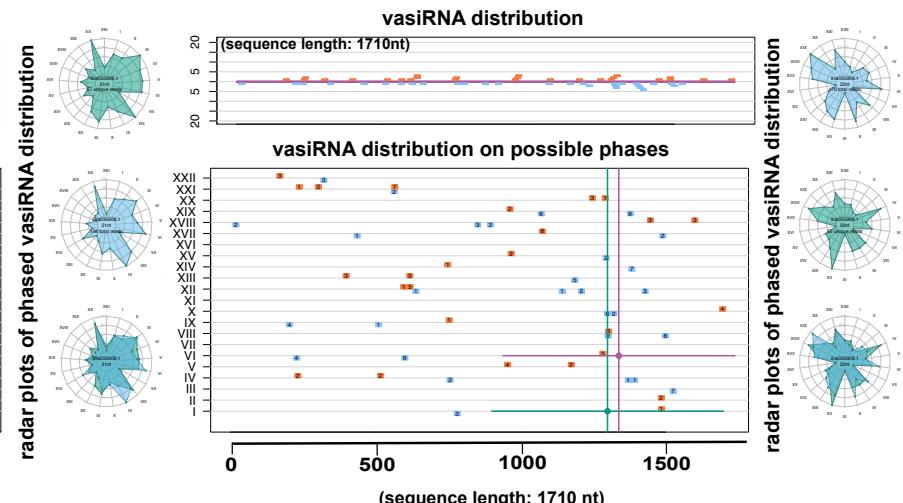
read length 22-nt

(d) **Oilseed Rape HSP70-1 (*BnaA09g05850D-1*)**
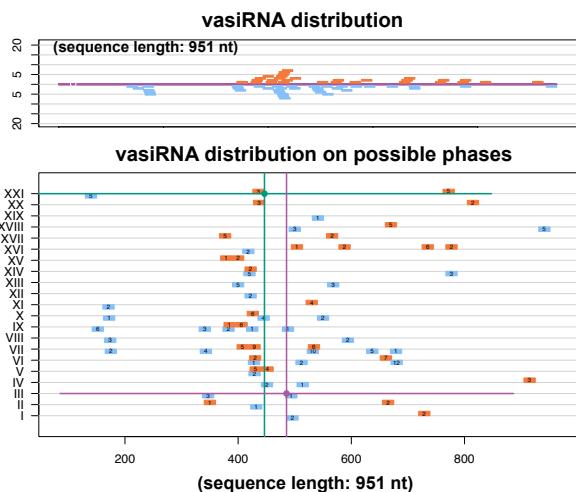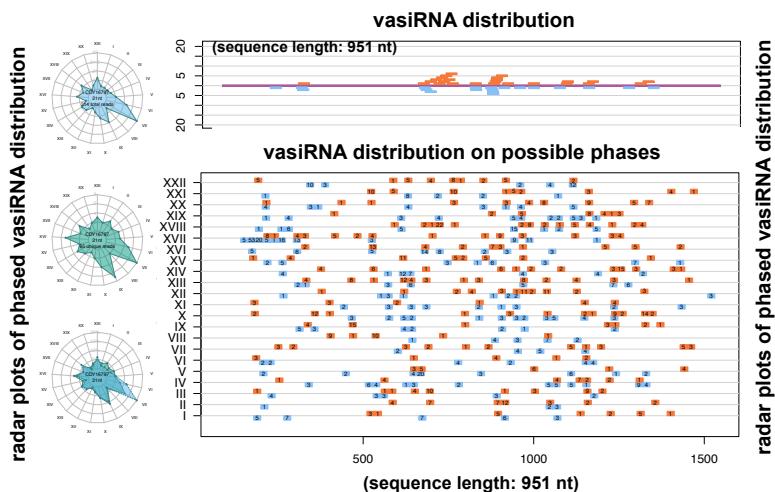
(e) **Turnip LHCB1.4 (*Bra005425.1*)**
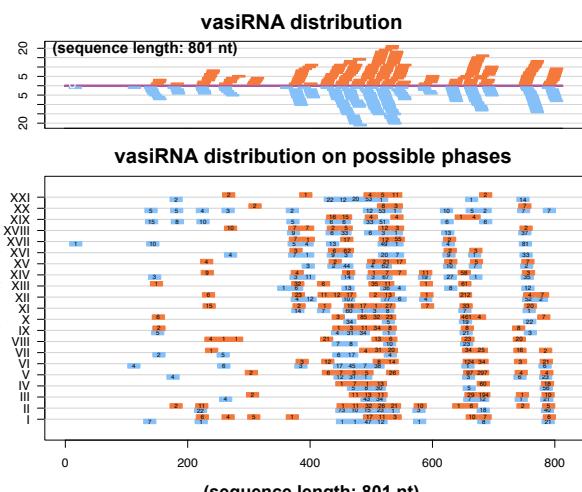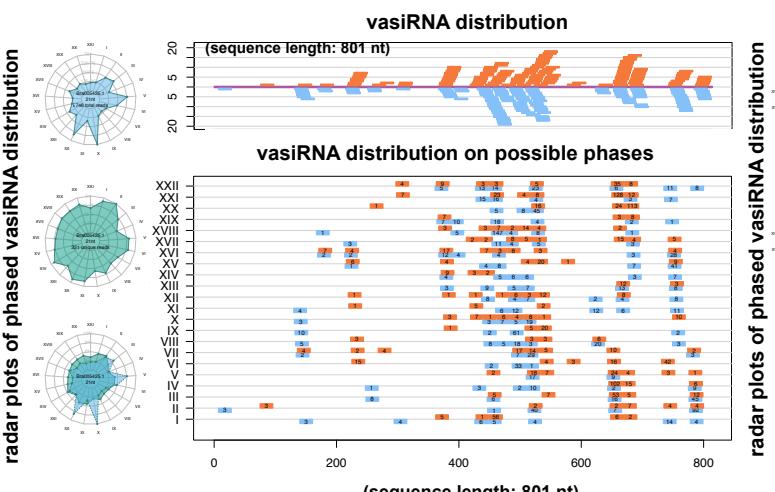
(f) **Oilseed Rape LHCB1.4 (*BnaA05g09410D-1*)**
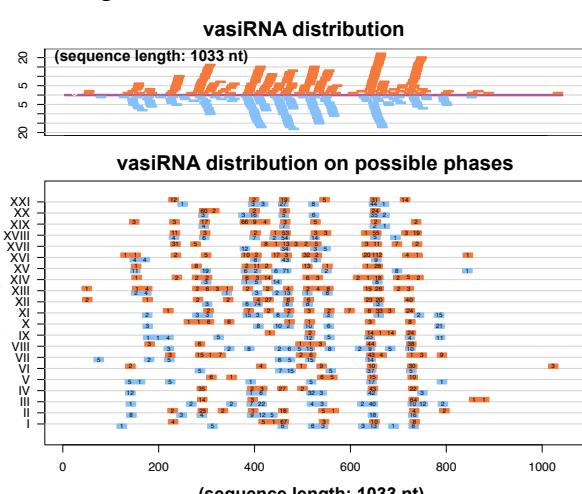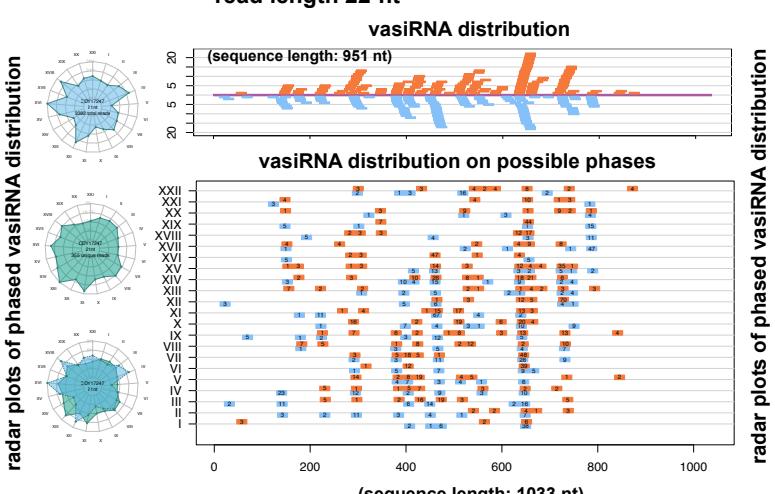
(g) Turnip SAP (*Bra020123.1*)

read length 21-nt

vasiRNA distribution

(sequence length: 753 nt)

vasiRNA distribution on possible phases

(sequence length: 753 nt)

read length 22-nt

vasiRNA distribution

(sequence length: 753 nt)

vasiRNA distribution on possible phases
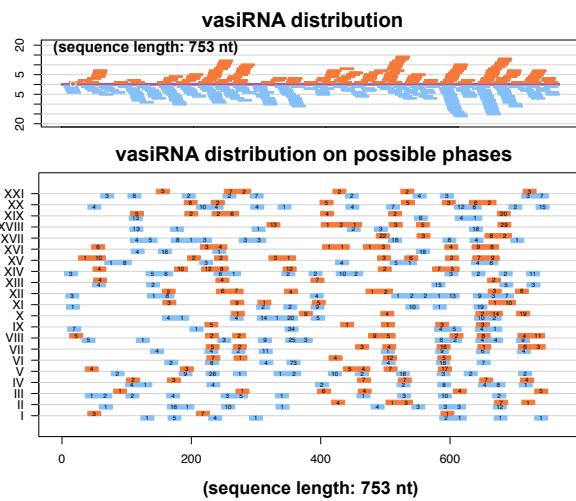
(sequence length: 753 nt)
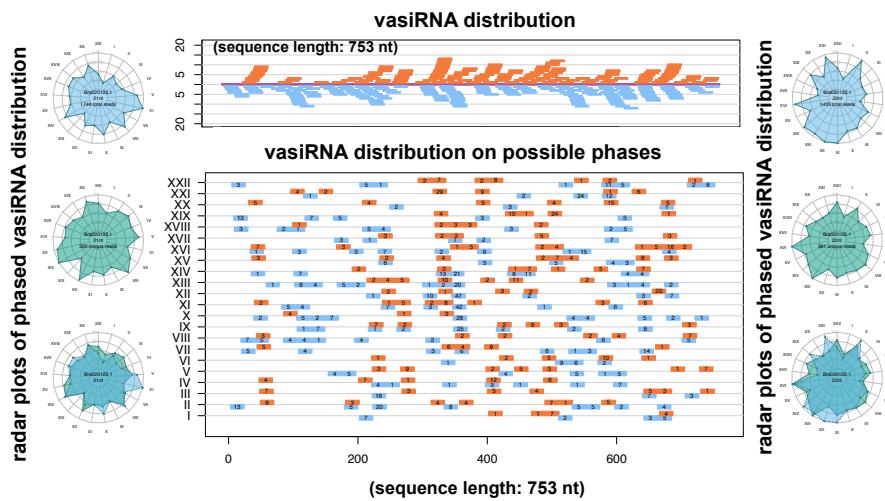
(h) Oilseed Rape SAP (*BnaA02g05040D-1*)

read length 21-nt

vasiRNA distribution

(sequence length: 1131 nt)

vasiRNA distribution on possible phases

(sequence length: 1131 nt)

read length 22-nt

vasiRNA distribution

(sequence length: 1131 nt)

vasiRNA distribution on possible phases
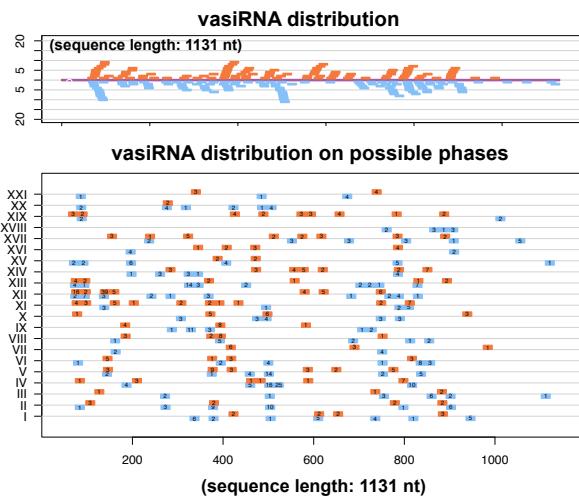
(sequence length: 1131 nt)
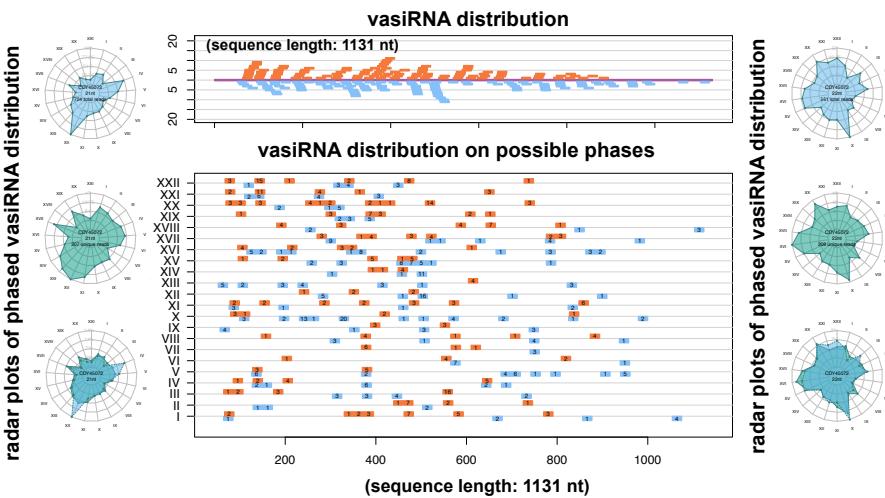
(i) Turnip FBA-1 (*Bra030303.1*)

read length 21-nt

vasiRNA distribution

(sequence length: 1162 nt)

vasiRNA distribution on possible phases

(sequence length: 1162 nt)

read length 22-nt

vasiRNA distribution

(sequence length: 1162 nt)

vasiRNA distribution on possible phases
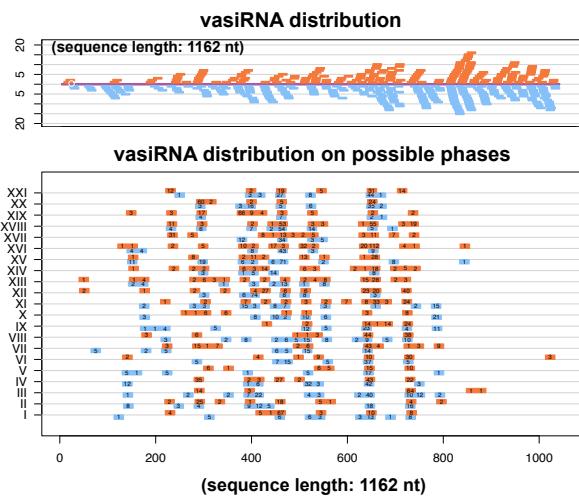
(sequence length: 1162 nt)
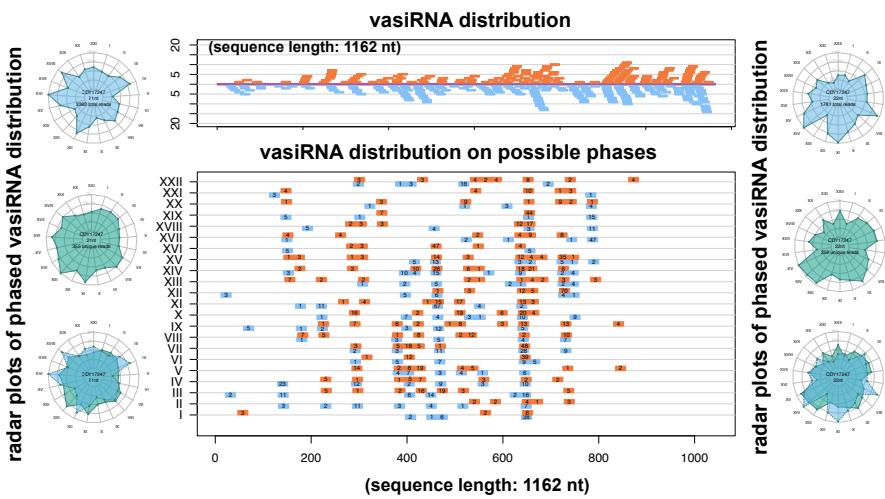
(j)   Oilseed Rape FBA-1 (*BnaA04g12130D-1*)

read length 21-nt
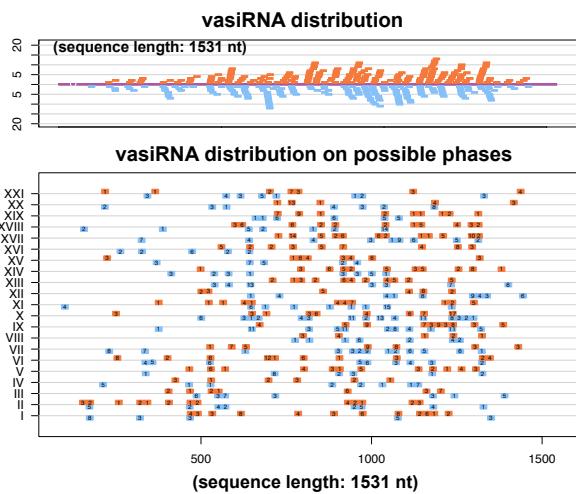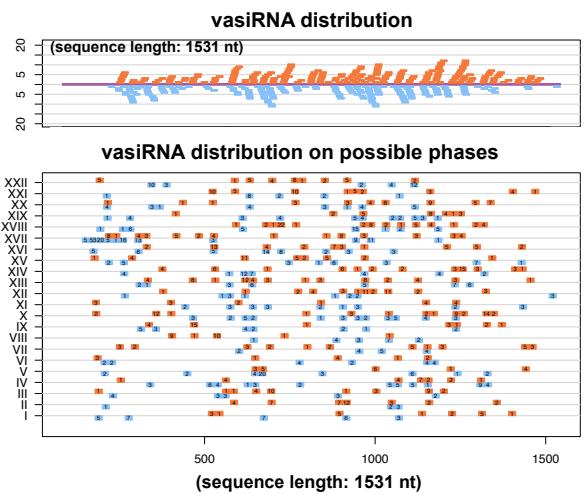

read length 22-nt

(k)   Turnip LHCA1 (*Bra003198.1*)

read length 21-nt
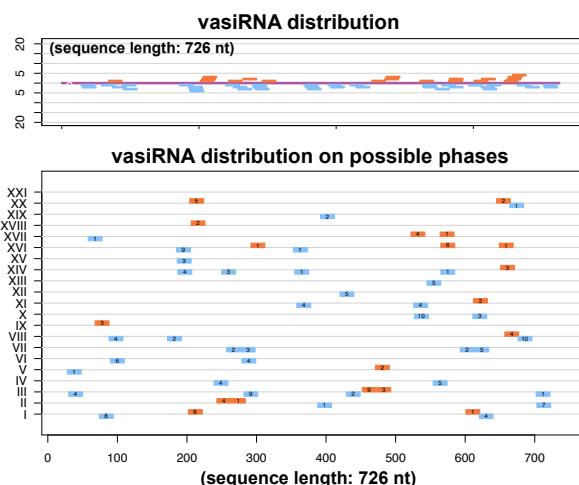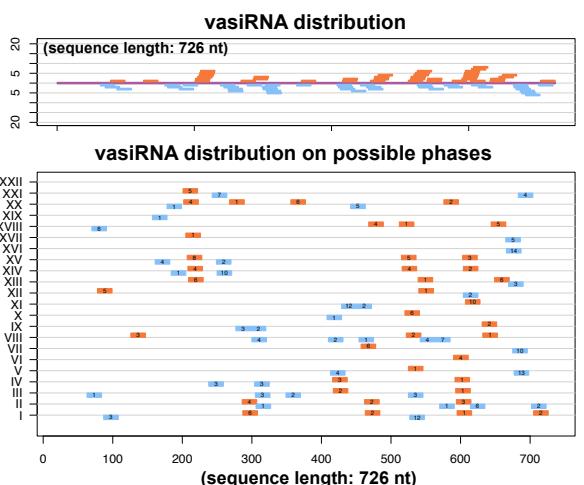
read length 22-nt

(l)   Oilseed Rape LHCA1 (*BnaA07g16530D-1*)

read length 21-nt
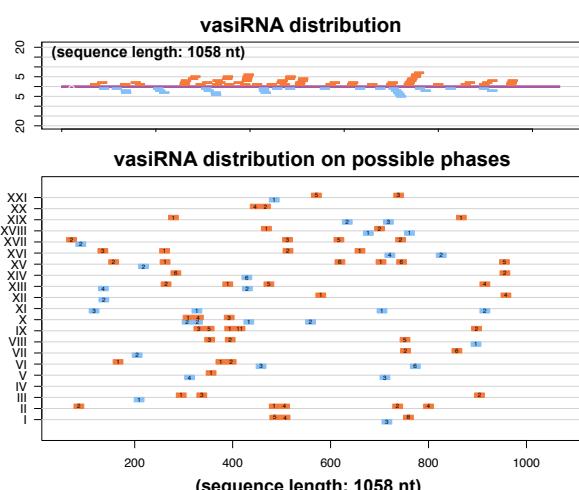
read length 22-nt
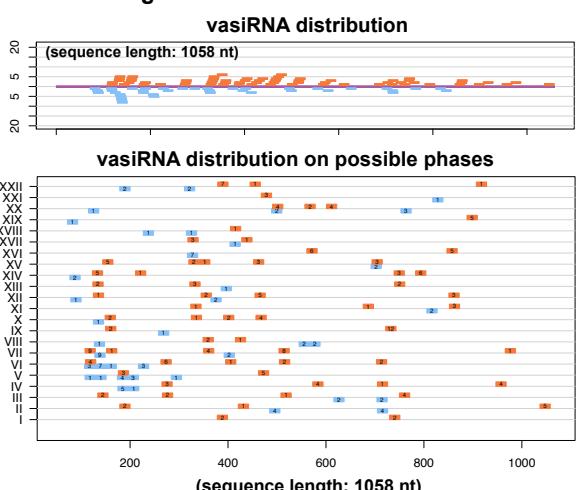
Fig. S6. **21- and 22-nt vasiRNAs Distribution and Phasing in *Arabidopsis thaliana*, Turnip and Oilseed Rape.** (**a**) vasiRNAs distribution along the whole Turnip RCA transcript (*Bra017055.1*, 1332 nt in length) and (**b**) Oilseed Rape RCA transcript (*BnaC04g46560D-1*, 1928-nt in length). (**c**) vasiRNAs from Turnip HSP70-1 transcript (*Bra035909.1*, 1710 nt in length) and (**d**) Oilseed Rape HSP70-1 transcript (*BnaA09g05850D-1*, 951-nt in length). (**e**) vasiRNAs distribution along the whole Turnip LHCB1.4 transcript (*Bra005425.1*, 801-nt in length) and (**f**) Oilseed Rape LHCB1.4 transcript (*Bna05g0941D-1*, 1033-nt in length). (**g**) vasiRNAs distribution along the whole Turnip SAP transcript (*Bra020123.1*, 753-nt in length) and **(h)** Oilseed Rape SAP transcript (*BnaA02g05040D-1*, 1131-nt in length). **(i)** vasiRNAs distribution along the whole Turnip FBA-1 transcript (*Bra030303.1*, 1162-nt in length) and (**j**) Oilseed Rape FAB-1 transcript (*BnaA04g12130D-1*, 1531-nt in length). (**k**) vasiRNAs distribution along the whole Turnip LHCA1 transcript (*Bra003198.1*, 726-nt in length) and **(l)** Oilseed Rape LHCA1 transcript (*BnaA07g16530D-1*, 1058-nt in length). Upper panels show unique 21- and 22-nt vasiRNAs distribution along the selected transcript. Number of unique reads at Y axis. Bottom plots represent vasiRNAs distribution along each possible phases at Y axis, i.e. I to XXI (21-nt long vasiRNAs) or I to XXII (22-nt long vasiRNAs). The phase numbered as "I" ends exactly at the 3' end of the transcript, and the others are shifted by one towards 5' end. Each line corresponding to the phase links the reads associated to it, along the transcript. The number in each read shows the number of replicates of the read that were found in the sequencing. Radar plots represent phased unique (top), redundant (middle) and the superimposition of the two (bottom) of 21-22-nt vasiRNAs.

Fig. S7. **Detection and quantification of LHCB1.3 transcripts. (a)** Detection of LHCB1.3 antisense transcript in Mock-inoculated (M) Turnip tissues. It reports gel-electrophoresis pattern of PCR products obtained using "RT_minus LHCB1.3_rev/RT_minus LHCB1.3_for" or "RT_minus LHCB1.3_rev/RT_minus LHCB1.3_for" combinations of oligonucleotides (lane 1 with products of 163 bp and 2 with products of 255 bp, respectively). In both cases oligonucleotide "RT_minus LHCB1.3_for" was used for strand-specific cDNA synthesis.

(**b**) Strategy used for quantitative detection of sense (in orange) or antisense (in blue) LHCB1.3 transcripts. Oligonucleotides "LHCB1.3_6b_for" or "LHCB1.3_770b_rev" were used respectively for cDNA synthesis of antisense or sense LHCB1.3 RNA transcripts, respectively. Both strand-specific cDNAs encompasses three regions (block 1, 2, 3 in the figure) were used for qPCR. Green and red arrows represent forward and reverse oligonucleotides, respectively (referred to the conventional 5'-3' orientation of the mRNA).

Quantification by qPCR on Turnip **(c)** and Oilseed Rape **(d)** of either sense (orange bars) or antisense (blue bars) LHCB1.3 transcripts corresponding to blocks 1, 2 and 3. Means were determined from three biological replicates. Data are the mean Log2 fold changes ± standard deviation of accumulation levels of LHCB1.3 transcripts in CaMV-infected relative to Mock-inoculated plant tissues (normalized to the expression of the constitutive gene UBC10). Asterisks indicate that the mean Log2 fold change is significantly different as determined by Student's test ("*"P < 0.05; "**"P < 0.01). For oligonucleotide sequences see Table S4.

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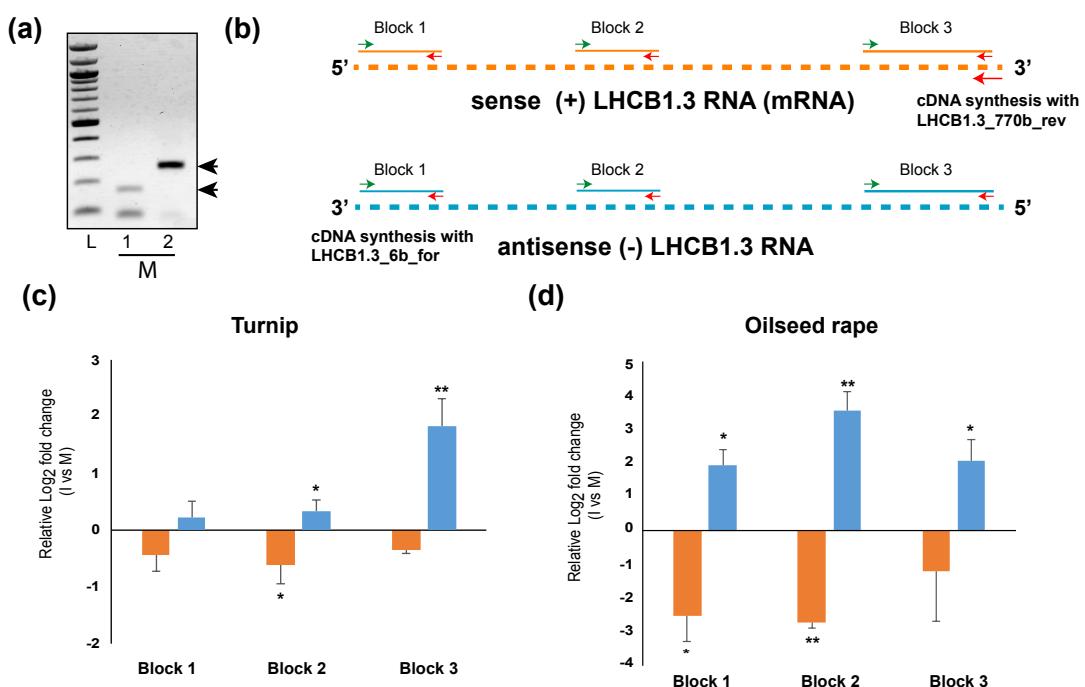
Table S1 Oligonucleotides used in this study.

Use	Primer name	Sequence (5' to 3')
qPCR oligonucleotides.	UBC10-R	ATGGAACACCTTCGTCCTAAA
	UBC10-F	GGGTCCTACAGACAGTCCTTAC
	DCL2-R	CAGTGTCGCAAGGGCTAAGA
	DCL2-F	TTTCAAGTACTACACGTACGTGGAAA
	DCL4-R	GCTGTTCAACCAAAGCCACA
	DCL4-F	GGGAAGACTCACATTGCTGT
	RDR1-R2	TGGGGACGATGTCTTGTTCG
	RDR1-F2	CGCCATCGTTCAGTTCACATC
	CP5-F	CGTGGGAGATAGCAAAGCTC
	CP5-R	TCTCCTGCAACCTCTCCTGT
	BraAGO2-F	AAACTGGCCTGAAGCTAACC
	BraAGO2-R	GACACCATCACGGAAGATCA
	BnaAGO2-F	TCGGAGCTATACGTTCACCA
	BnaAGO2-R	CACAACATCCATCCCTTGC
	BraLHCB1.3-F	AGACTACGGGTGGGACACAG
	BraLHCB1.3-R	TCCGAACTTGACTCCATTCC
	BnaLHCB1.3-F	TTCCCAGGAGACTACGGATG
	BnaLHCB1.3-R	ATCAAGTCCTCCTTCGCTGA
	BraSAP-F	TGGGTACGGAATCTCCAAAG
	BraSAP-R	GAAAACTTGACCGACGGTGT
	BnaSAP-F	AGCACGGACGAAGAAGAAGA
	BnaSAP-R	TACCGACATCGACCATGAAA
	BraHSP70.1-F	ACCAGCGTTGGAGAGAAGAA
	BraHSP70.1-R	GGGTTTCCGGTAATGTCCTT
	BnaHSP70.1-F	TACCATTCCGACCAAGAAGG
	BnaHSP70.1-R	CGTTGGTGATGGTGATCTTG
	RCA-F	GTCCAGCTCCCAGGAATGTA
	RCA-R	ACCCTTGCAGACACCGATAC
RT-PCR oligonucleotides.	RCA-R	ACCCTTGCAGACACCGATAC
	T7 RCA-F	**TAATACGACTCACTATAG**GGGAAAGAAAGTTGTAACCG
	HSP70-R	TTAGTCGACCTCTTCGATCTTAGG
	T7 HSP70-F	**TAATACGACTCACTATAG**GGAATCCCTCCAGCTCCACG
	LHCB1.3-R	GCAAGTCCTCGGCCTCTCCC
	T7 LHCB1.3-F	**TAATACGACTCACTATAG**GGCCGTGTGACAATGAGGAAGAC
siRNA guide strands	RCA_121	CUUGGCUGAGGUACUCAUAGG
	RCA_1	CUUGGCUGAGGUACUCAUAGGA
	RCA_2	UGUCGUUGGUGAUGGUGAUCUU
	HSP70_1	AUCAUGUUGUCCAAGUUGUACU
	HSP70_221	AGCUCCUUGGUACAUCUUGGC
	HSP70_2	AGCUCCUUGGUACAUCUUGGCA
	LHCB1.3_121	AACUCUCCGGUAAGGUAGCUC
	LHCB1.3_1	AACUCUCCGGUAAGGUAGCUCG
	LHCB1.3_2	UCUCGGGAUCGGCUGAGAGACC
siRNA passenger strands	RCA_121plus	UAUGAGUACCUCAGCCAAGGU
	RCA_1 PLUS	CUAUGAGUACCUCAGCCAAGGU
	RCA_2 PLUS	UACAACUUGGACAACAUGAUGG
	HSP70_1 PLUS	GAUCACCAUCACCAACGACAAG
	HSP70_221 PLUS	CAAGAUGUACCAAGGAGCUGG
	HSP70_2 PLUS	CCAAGAUGUACCAAGGAGCUGG
	LHCB1.3_121PLUS	GCUACCUUACCGGAGAGUUCC
	LHCB1.3_1 PLUS	AGCUACCUUACCGGAGAGUUCC
	LHCB1.3_2 PLUS	UCUAUCAGCCGAUCCCGAGACC
qPCR oligonucleotides (sense and antisense LHCB1.3 RNAs).	LHCB1.3_6b_for	CGCCTCAACAATGGCTCTCT
	LHCB1.3_93b_rev	TCTTCCCCATTGTCACACGG
	LHCB1.3_277b_for	GCAAGGAACCGTGAGCTAGA
	LHCB1.3_367b_rev	GCCTCTCCGAACTTGACTCC
	LHCB1.3_625b_for	GCGGAGTTGAAGGTGAAGGA
	LHCB1.3_770b_rev	AAGTTGGTAGCAAAGGCCCA
	RT_minus LHCB1.3_for	GGAGACTACGGATGGGACACCGC
	RT_minus LHCB1.3_rev	CCGCCCTCCCGAACTTGACTCC
	RT_minus LHCB1.3C rev	AGCCAAGATGCTCTGAGCGTG

(In bold and underlined the T7 promoter sequence).

Table S2. Statistic of small RNAs from Arabidopsis and Brassica sp. related to Fig. 1b.

			read	Match with genome	Match with CaMV	read	Match with genome	Match with CaMV
Arabidopsis thaliana	mock	Raw read	-					
		Adapter removed						
		Filter by sequence length (20-25 nt)	3,934,819	2,941,913	388	1,017,935	633,550	168
	infected	Raw read	-					
		Adapter removed						
		Filter by sequence length (20-25 nt)	4,267,613	1,462,102	1227067	884,510	585,673	25705
Brassica rapa	mock	Raw read	17,269,029					
		Adapter removed	17,179,167					
		Filter by sequence length (20-25 nt)	15,718,199	9,972,961	1221	1,829,782	873,898	465
	infected	Raw read	13,193,022					
		Adapter removed	13,138,090					
		Filter by sequence length (20-25 nt)	11,500,370	5,171,176	1991562	2,257,515	1,054,620	44692
Brassica napus	mock	Raw read	16,598,214					
		Adapter removed	16,440,452					
		Filter by sequence length (20-25 nt)	14,873,062	11,102,831	17077	311,724	93,344	391
	infected	Raw read	13,393,049					
		Adapter removed	13,354,189					
		Filter by sequence length (20-25 nt)	9,684,196	6,887,845	786578	1,852,121	1,102,842	37848

Table S3. χ2 test and fold change evaluation for unique read counts, grouped by length (nt). Related to Fig. 1d.

Plant	Read length	Mock raw counts	Infected raw counts	Mock ppm	Infected ppm	Fold Change (FC)	Logarithmic Fold Change (log2FC)	Adjusted p-value
Arabidopsis	20	57737	40555	5672	4585	0,81	-0,31	< 2.2E-16
Arabidopsis	21	311193	155490	30571	17579	0,58	-0,80	< 2.2E-16
Arabidopsis	**22**	**81689**	**128450**	**8025**	**14522**	**1,81**	**0,86**	**< 2.2E-16**
Arabidopsis	23	156436	87833	15368	9930	0,65	-0,63	< 2.2E-16
Arabidopsis	24	352440	428240	34623	48415	1,40	0,48	< 2.2E-16
Arabidopsis	25	58440	43943	5741	4968	0,87	-0,21	< 2.2E-16
Turnip	20	64372	107232	3518	4750	1,35	0,43	< 2.2E-16
Turnip	21	547672	480061	29931	21265	0,71	-0,49	< 2.2E-16
Turnip	**22**	**186638**	**396600**	**10200**	**17568**	**1,72**	**0,78**	**< 2.2E-16**
Turnip	23	248064	339756	13557	15050	1,11	0,15	< 2.2E-16
Turnip	24	760567	867924	41566	38446	0,92	-0,11	< 2.2E-16
Turnip	25	22470	65942	1228	2921	2,38	1,25	< 2.2E-16
Oilseed rape	20	12625	174765	4050	9436	2,33	1,22	< 2.2E-16
Oilseed rape	21	66089	396376	21201	21401	1,01	0,01	0,051078709
Oilseed rape	**22**	**31958**	**339733**	**10252**	**18343**	**1,79**	**0,84**	**< 2.2E-16**
Oilseed rape	23	51001	299987	16361	16197	0,99	-0,01	0,051467887
Oilseed rape	24	143271	535096	45961	28891	0,63	-0,67	< 2.2E-16
Oilseed rape	25	6780	106163	2175	5732	2,64	1,40	< 2.2E-16

Cell color legend	
	Significant result
	FC < 0.66 equivalent to log2FC < -0.58
	FC > 1.5 equivalent to log2FC > 0.58
	Adjusted p-value < 0.05

N.B. Adjusted p-value < 2.2E-16 is a value close to 0, limited by computable floating point precision

Table S4. Output of unique 21-nt and 22-nt vasiRNA analysis in mock-inoculated and CaMV-infected tissues, referred to each gene in Table 1.

NAME	A. thaliana	Mock 21	Mock 22	Mock tot	CaMV 21	CaMV 22	CaMV tot	Turnip	Mock 21	Mock 22	Mock tot	CaMV 21	CaMV 22	CaMV tot	Oilseed rape	Mock 21	Mock 22	Mock tot	CaMV 21	CaMV 22	CaMV tot
SAP	AT5G20700.1	13	6	**19**	120	152	**272**	Bra020123.1	13	3	**16**	320	281	**601**	BnaA02g05040D-1	4	3	**7**	207	208	**415**
ATPase	AT2G18193.1	0	0	**0**	22	50	**72**	Bra024477.1	1	0	**1**	79	42	**121**	BnaA02g05040D-1	0	0	**0**	65	37	**102**
ABCG36	AT1G59870.1	2	1	**3**	43	70	**113**	Bra003527.1	2	2	**4**	80	100	**180**	BnaA07g19610D-1	1	0	**1**	118	112	**230**
CP5	AT1G64720.1	7	4	**11**	50	57	**107**	Bra017435.1	0	0	**0**	33	36	**69**	BnaC09g20830D-1	1	0	**1**	33	64	**97**
ACT2	AT3G18780.1	3	3	**6**	27	39	**66**	Bra037560.1	5	1	**6**	112	161	**273**	BnaA01g26500D-1	1	2	**3**	450	764	**1214**
RPT2	AT2G30520.1	2	2	**4**	36	61	**97**	Bra018309.1	2	0	**2**	49	56	**105**	BnaA05g11930D-1	0	0	**0**	25	52	**77**
CAT3	AT1G20620.4	10	5	**15**	151	215	**366**	Bra016459.1	0	0	**0**	51	56	**107**	BnaA08g21730D-1	0	0	**0**	67	92	**159**
GUN5	AT5G13630.2	5	1	**6**	54	99	**153**	Bra006208.1	5	4	**9**	45	56	**101**	BnaA03g04440D-1	1	0	**1**	78	82	**160**
FBA1	AT2G21330.2	2	3	**5**	29	39	**68**	Bra030303.1	7	2	**9**	405	313	**718**	BnaA04g12130D-1	2	0	**2**	361	360	**721**
HSP70-1	AT5G02500.1	5	7	**12**	128	117	**245**	Bra035909.1	5	2	**7**	57	55	**112**	BnaA09g05850D-1	0	1	**1**	65	44	**109**
LHCA1	AT3G54890.2	6	6	**12**	21	40	**61**	Bra003198.1	4	5	**9**	55	79	**134**	BnaA07g16530D-1	1	0	**1**	87	104	**191**
LHCB1.3	AT1G29930.1	29	27	**56**	453	365	**818**	Bra010807.1	77	27	**104**	658	499	**1157**	BnaC03g59520D-1	7	1	**8**	762	606	**1368**
LHCB1.4	AT2G34430.1	12	3	**15**	170	124	**294**	Bra005425.1	21	6	**27**	332	235	**567**	BnaA05g09410D-1	1	0	**1**	355	259	**614**
LOS1	AT1G56070.3	9	6	**15**	47	60	**107**	Bra020542.1	5	0	**5**	113	130	**243**	BnaA02g31580D-1	1	0	**1**	77	63	**140**
NFXL1	AT1G10170.1	0	0	**0**	108	120	**228**	Bra018458.1	0	0	**0**	38	33	**71**	BnaC08g14030D-1	0	0	**0**	81	72	**153**
RCA	AT2G39730.3	17	13	**30**	36	49	**85**	Bra017055.1	14	10	**24**	91	107	**198**	BnaC04g46560D-1	1	1	**2**	333	392	**725**

Table S5. Microarray comparison of expression profiles of CP5, HSP70-1 and SAP. CaMV-infected *Arabidopsis thaliana* Col-0, dcl2-dcl4, dcl2-dcl3 and dcl4-dcl3 double mutants were compared to those of control plants. In orange and blue, the Log2fold that change greater than two folds. *CATMA5A01580 is used in substitution to the CATMA5A01540 for HSP70-1 because the latter is not available for this combination.

Comparison	ID microarray	Log2 fold change	Fold change	Gene	Description
Col-0_CaMV_vs_Col-0_Mock	CATMA1A54045	-0,11	0,927230546	AT1G64720	CP5
	CATMA4A01540	-1,23	0,425431865	AT5G02500	HSP70-1
	CATMA5A19230	-2,85	0,138696184	AT5G20700	SAP
dcl2_dcl4_CaMV_vs_Col-0_CaMV	CATMA1A54045	2,34	5,052509047	AT1G64720	CP5
	CATMA5A01580*	0,83	1,782620992	AT5G02500	HSP70-1
	CATMA5A19230	2,00	4,011105744	AT5G20700	SAP
dcl2_dcl3_CaMV_vs_Col-0_CaMV	CATMA1A54045	0,87	1,823866331	AT1G64720	CP5
	CATMA4A01540	1,06	2,077718207	AT5G02500	HSP70-1
	CATMA5A19230	1,33	2,507066041	AT5G20700	SAP
dcl3_dcl4_CaMV_vs_Col-0_CaMV	CATMA1A54045	0,68	1,601029621	AT1G64720	CP5
	CATMA4A01540	-0,31	0,804408371	AT5G02500	HSP70-1
	CATMA5A19230	1,30	2,46058269	AT5G20700	SAP