SUPPLEMENTARY DATA

Target	Primer	Primer Sequences	Annealing	RT-PCR
transcripts	pair	$5' \rightarrow 3'$	temperature	product size
*	•		(°C)	(bp)
S-RNases	C2f	AACTTTACRATYCAYGRCTTTG	47,4	~420
(C2-C5	C5r	RAAACATATRCCWAYCTC		
fragment)				
S-RNases	C2f	see above	47,4	~280
(C2-C4	C4r	CARAAGATCAAACTTRTCT		
fragment)				
NnSR1	NnRS1f	GACTGTGGAGACATTTACGA	63	313
	NnRS1r	TAGGATACGCTTGAGTAACTGCT		
NnSR2	NnRS2f	TTAACTGCGAGTCAAATAAG	53,9	332
	NnRS2r	AATGCAGTTGAGGTTAGGA		
NE	NIE£		50	202
NE	NEI		38	203
	NEr	ACAAGIGCCAIGIIIIICCCAIICAIG		
Actin	Actinf	TGGAGAAGATATGGCATCATAC	55 7	840
	Actinr	CTGGAAGGTGCTGAGGGAAG	22,1	010
	1 1001111			

TABLE S1. Primer pairs and PCR conditions used in this study.

FIG. S1. Specificity of pNnSR1 recognition by antisera. The antisera against BSA-pNnSR1 and KLH-pNnSR1 conjugates were assayed in their ability to recognize pNnSR1. (A) Anti BSA-pNnSR1 serum was assayed by immunodot. One µg of KLH, BSA, KLH-pNnSR1 and BSA-pNnSR1 was dotted onto nitrocellulose membrane and probed with anti B-pNnSR1 serum diluted 1:5,000. Preimmune control is shown. (B) Anti KLH-pNnSR1 serum was assayed by immunoblot. Two µg of each BSA and BSA-pNnSR1 were run by SDS-PAGE, blotted onto nitrocellulose filter and probed with anti KLH-pNnSR1 serum diluted 1:5000.



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FIG. S2. Expression of class III *NnSR1* transcript induced by Pi-deprivation in *Nicotiana alata* roots. RT-PCR amplification from roots exposed to 14 days of Pi-starvation. Degenerate primers designed from C2 and C4 conserved domains of *Nicotiana alata S-RNases* were used. A representative gel and the statistical analysis are shown. Signal intensity values represent the mean \pm SEM of three independent experiments. Data were analyzed using the *t*-test. ***, high significant differences (*P*< 0.001). Pi–, Pi deficient medium; Pi+, Pi sufficient medium.



FIG. S3. Expression of class III *non-S-RNase* transcripts induced by Pi-deprivation in *Nicotiana alata* roots. RT-PCR amplification from roots exposed to 14 days of Pi-starvation is shown. Specific primers for *NnSR1* and *NnSR2* amplification were used. Pi–, Pi deficient medium; Pi+, Pi sufficient medium.



FIG. S4. Amino acid sequence alignment of RNases from *Nicotiana alata*. The C2-C5 region of the functional S₇₀-RNase, the non-S-RNases NnSR1 and NnSR2, and the S-like RNase NE is shown. Distinctive amino acid patterns for class III S-RNase and non-S-RNases (grey) and for class I S-like RNases (pink) were shaded (Vieira *et al.*, 2008). RNase conserved domains are underlined. Identical amino acids (*) and conservative substitutions (. :) are shown.

	<u>C2</u>
S-RNase S70-RNase	NFTIHGLWPDDQHG-MLNDCRKTFTKLSDPREMKELDDRWPDLNRSPNDAKKEQSFWR
Non S-RNase NnSR1	NFTIHGLWPDEQHG-MLNDCGETFTKLREPREKKELDDRWPDLKRSRSDAQEVQSFWE
NnSR2	NFTIHGLWPDKQNT-MLINCES-NKYTDIKDPRKCKQLEYYWPDLTAKVGDIKKHQGFWK
S-like RNase NE	DFGIHGLWPNNNDGSYPSNCDSNSPYDQSQVSDLISRMQQNWPTLACPSGTGSAFWS
	:* *****:.:. :* . ::: ** ***
	<u>C3</u> <u>C4</u>
S-RNase S70-RNase	YEYNKHGTCCTELYNQDAYFDLAKNLKDRFDLLRILRNQGIIP-GSAHTVDKISEAVRAV
Non S-RNase NnSR1	YEYNKHGTCCTELYDQAAYFDVAKNLKDKFDLLRNLKNEGIIP-GSTYTVDEIAEAIRAV
NnSR2	YEFNKHGTCSKELYNQDAYFDLAIKLKNKFDLLSTLGNQGIIP-GKIRTVKNVEDAIEAV
S-like RNase NE	HEWEKHGTCSESIFDQHGYFKKALDLKNQINLLEILQGAGINPDGGFYSLNSIKNAIRSA
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	<u>C5</u>
S-RNase S70-RNase	TKAYPNLNCVGDPQKTLELSEIGICF
Non S-RNase NnSR1	TPAYPNLNCVGDPQKILELSEIGICF
NnSR2	TTKVPNLNCIGDSRWTMELLEIGICF
S-like RNase NE	IGYTPGIECNVDDSGNSQLYQVYICV
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FIG. S5. Expression of NnSR1 in roots, sepals and petals of *Nicotiana alata* exposed to 14 days of Pi-limitation. The anti BSA-pNnSR1 serum was used as probe. Thirty μ g of protein were loaded in each lane. The result is representative of three independent experiments. Pi–, Pi deficient medium; Pi+, Pi sufficient medium.

