SUPPLEMENTARY DATA

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

Target transcripts	Primer pair	Primer Sequences 5′→ 3′	Annealing temperature (ºC)	RT-PCR product size (bp)
S-RNases (C2-C5 fragment)	C2f C5r	AACTTTACRATYCAYGRCTTTG RAAACATATRCCWAYCTC	47,4	~420
S-RNases (C2-C4 fragment)	C2f C4r	see above CARAAGATCAAACTTRTCT	47,4	~280
NnSR1	NnRS1f NnRS1r	GACTGTGGAGACATTTACGA TAGGATACGCTTGAGTAACTGCT	63	313
NnSR2	NnRS2f NnRS2r	TTAACTGCGAGTCAAATAAG AATGCAGTTGAGGTTAGGA	53,9	332
NE	NEf NEr	ATCCATGGACTTTGGCCTAA ACAAGTGCCATGTTTTTCCCATTCATG	58	203
Actin	Actinf Actinr	TGGAGAAGATATGGCATCATAC CTGGAAGGTGCTGAGGGAAG	55,7	840

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.

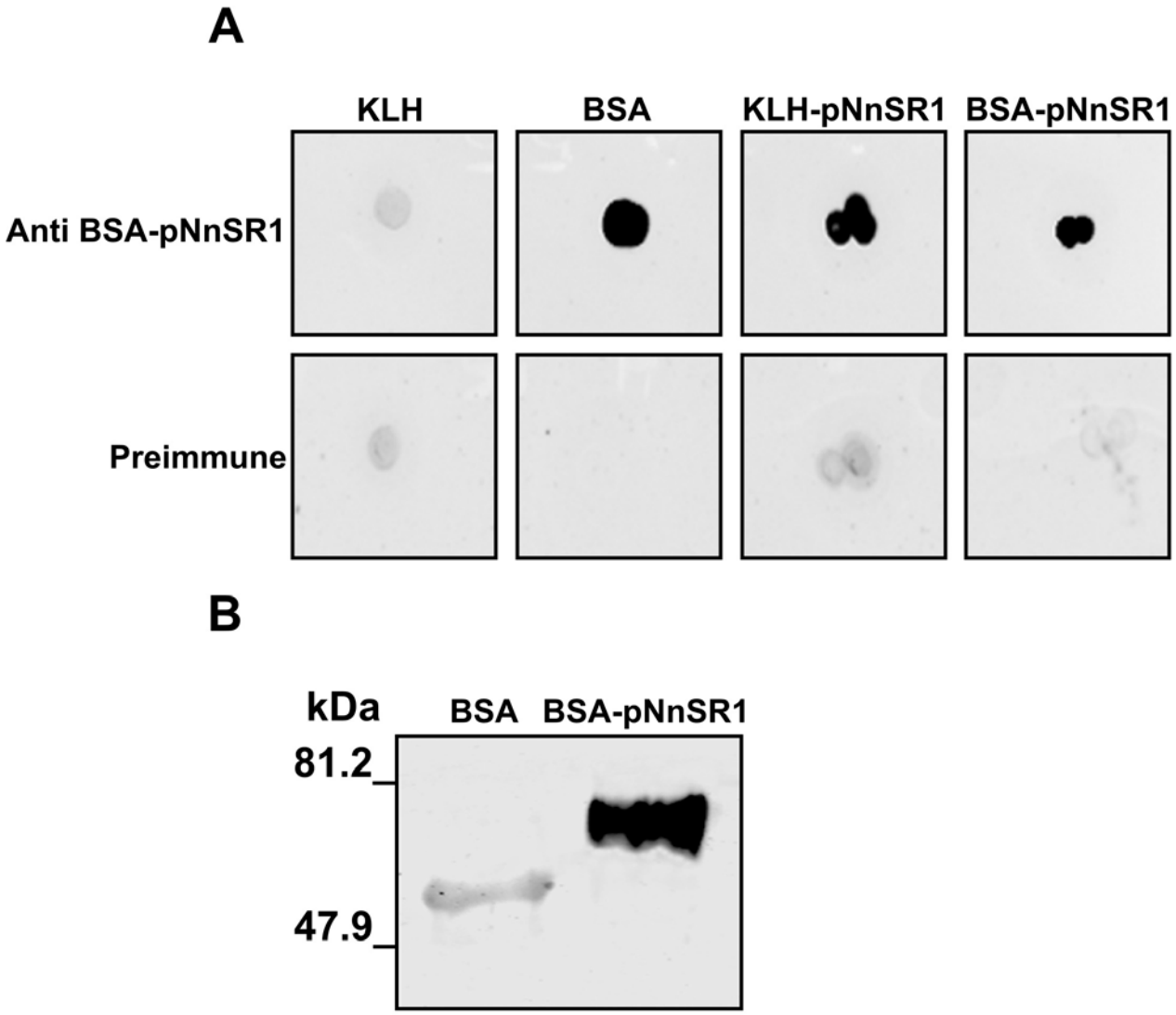

FIG. S2. Expression of class III *NnSR1* transcript induced by Pi-deprivation in *Nicotiana alata* roots. RT-PCR amplification from roots exposed to 14 days of Pi-starvation. Degenerate primers designed from C2 and C4 conserved domains of *Nicotiana alata S-RNases* were used. A representative gel and the statistical analysis are shown. Signal intensity values represent the mean ± 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.

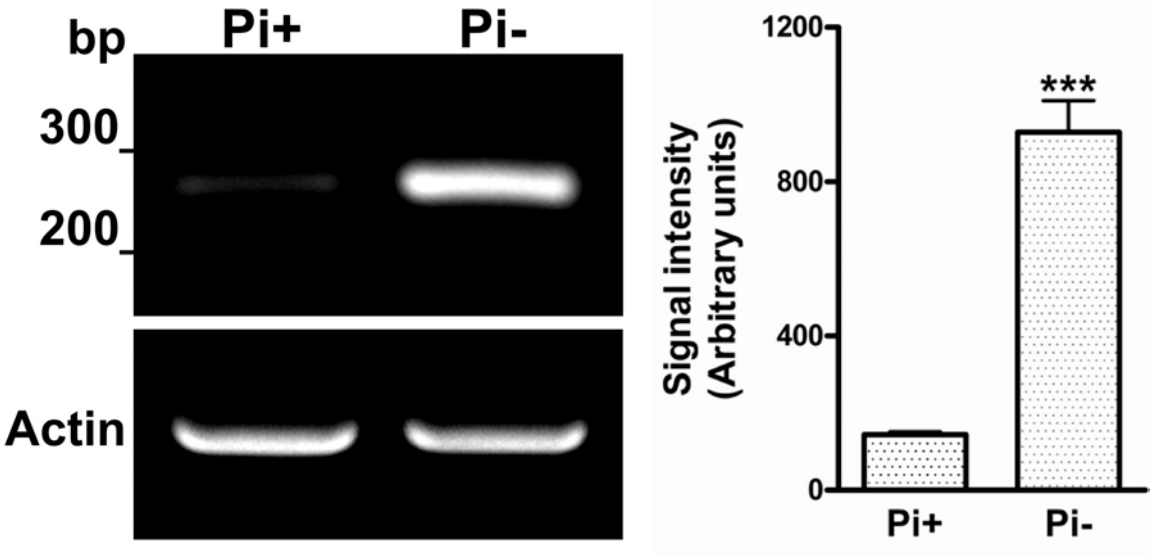

FIG. S3. Expression of class III *non-S-RNase* transcripts induced by Pi-deprivation in *Nicotiana alata* roots. RT-PCR amplification from roots exposed to 14 days of Pi-starvation is shown. Specific primers for *NnSR1* and *NnSR2* amplification were used. Pi–, Pi deficient medium; Pi+, Pi sufficient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

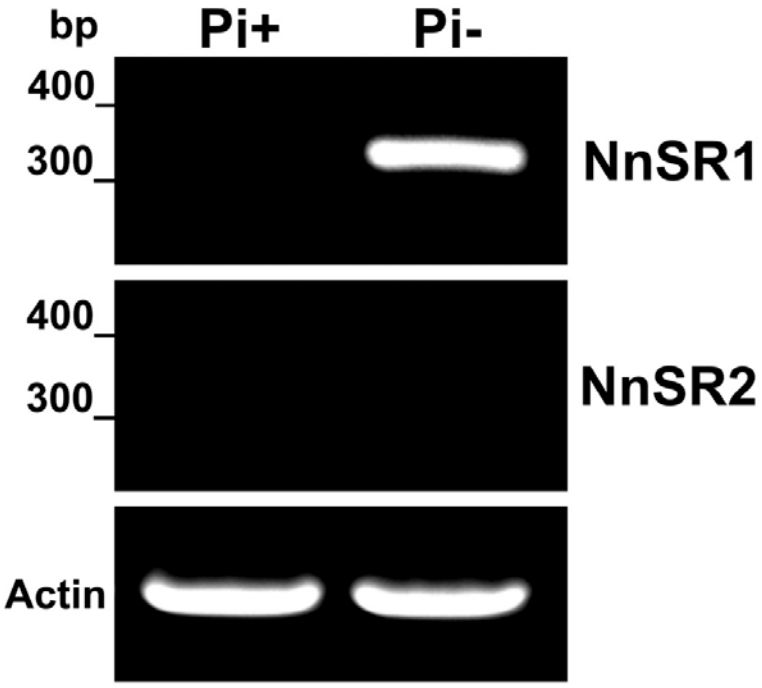

FIG. S4. Amino acid sequence alignment of RNases from *Nicotiana alata*. The C2-C5 region of the functional S_{70}-RNase, the non-S-RNases NnSR1 and NnSR2, and the S-like RNase NE is shown. Distinctive amino acid patterns for class III S-RNase and non-S-RNases (grey) and for class I S-like RNases (pink) were shaded (Vieira *et al*., 2008). RNase conserved domains are underlined. Identical amino acids (*) and conservative substitutions (. :) are shown.

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 µg of protein were loaded in each lane. The result is representative of three independent experiments. Pi–, Pi deficient medium; Pi+, Pi sufficient medium.