SUPPLEMENTARY DATA

FIG S1. Small RNA length distribution for four sequenced libraries.

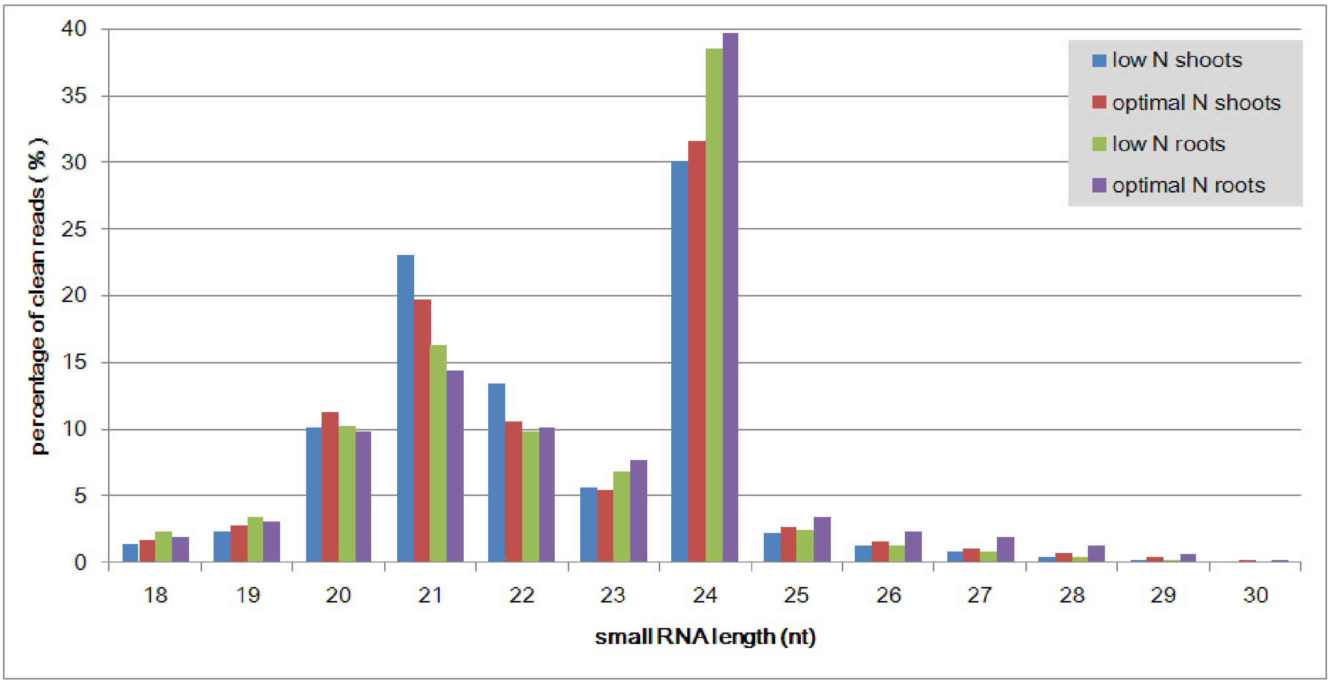
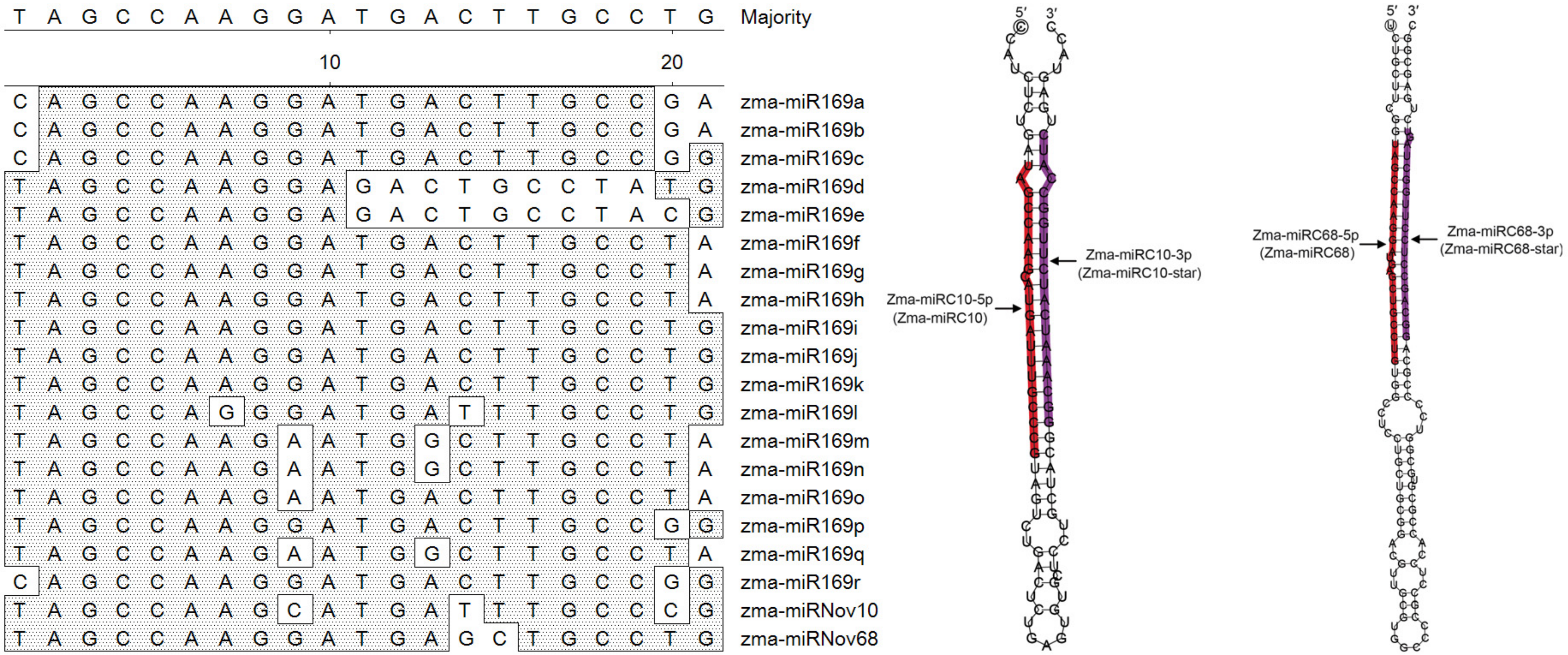


FIG. S2. Multiple alignments of known and two new zma-miR169 species, miRC10 and miR68, along with their hairpin secondary structure precursors. Note: all nucleotides ‘U’ in miRNAs were converted into ‘T’ for alignment by using DNAstar DNA sequence alignment tools.



									10										20		
T	A	G	C	C	A	A	G	G	A	T	G	A	C	T	T	G	C	C	T	G	Majority
C	A	G	C	C	A	A	G	G	A	T	G	A	C	T	T	G	C	C	G	A	zma-miR169a
C	A	G	C	C	A	A	G	G	A	T	G	A	C	T	T	G	C	C	G	A	zma-miR169b
C	A	G	C	C	A	A	G	G	A	T	G	A	C	T	T	G	C	C	G	G	zma-miR169c
T	A	G	C	C	A	A	G	G	A	G	A	C	T	G	C	C	T	A	T	G	zma-miR169d
T	A	G	C	C	A	A	G	G	A	G	A	C	T	G	C	C	T	A	C	G	zma-miR169e
T	A	G	C	C	A	A	G	G	A	T	G	A	C	T	T	G	C	C	T	A	zma-miR169f
T	A	G	C	C	A	A	G	G	A	T	G	A	C	T	T	G	C	C	T	A	zma-miR169g
T	A	G	C	C	A	A	G	G	A	T	G	A	C	T	T	G	C	C	T	A	zma-miR169h
T	A	G	C	C	A	A	G	G	A	T	G	A	C	T	T	G	C	C	T	G	zma-miR169i
T	A	G	C	C	A	A	G	G	A	T	G	A	C	T	T	G	C	C	T	G	zma-miR169j
T	A	G	C	C	A	A	G	G	A	T	G	A	C	T	T	G	C	C	T	G	zma-miR169k
T	A	G	C	C	A	G	G	G	A	T	G	A	T	T	T	G	C	C	T	G	zma-miR169l
T	A	G	C	C	A	A	G	A	A	T	G	G	C	T	T	G	C	C	T	A	zma-miR169m
T	A	G	C	C	A	A	G	A	A	T	G	G	C	T	T	G	C	C	T	A	zma-miR169n
T	A	G	C	C	A	A	G	A	A	T	G	A	C	T	T	G	C	C	T	A	zma-miR169o
T	A	G	C	C	A	A	G	G	A	T	G	A	C	T	T	G	C	C	G	G	zma-miR169p
T	A	G	C	C	A	A	G	A	A	T	G	G	C	T	T	G	C	C	T	A	zma-miR169q
C	A	G	C	C	A	A	G	G	A	T	G	A	C	T	T	G	C	C	G	G	zma-miR169r
T	A	G	C	C	A	A	G	C	A	T	G	A	T	T	T	G	C	C	C	G	zma-miRNov10
T	A	G	C	C	A	A	G	G	A	T	G	A	G	C	T	G	C	C	T	G	zma-miRNov68

FIG S3. Overview of the phenotype for nitrogen deficiency and hydroponic culture system used in this study.

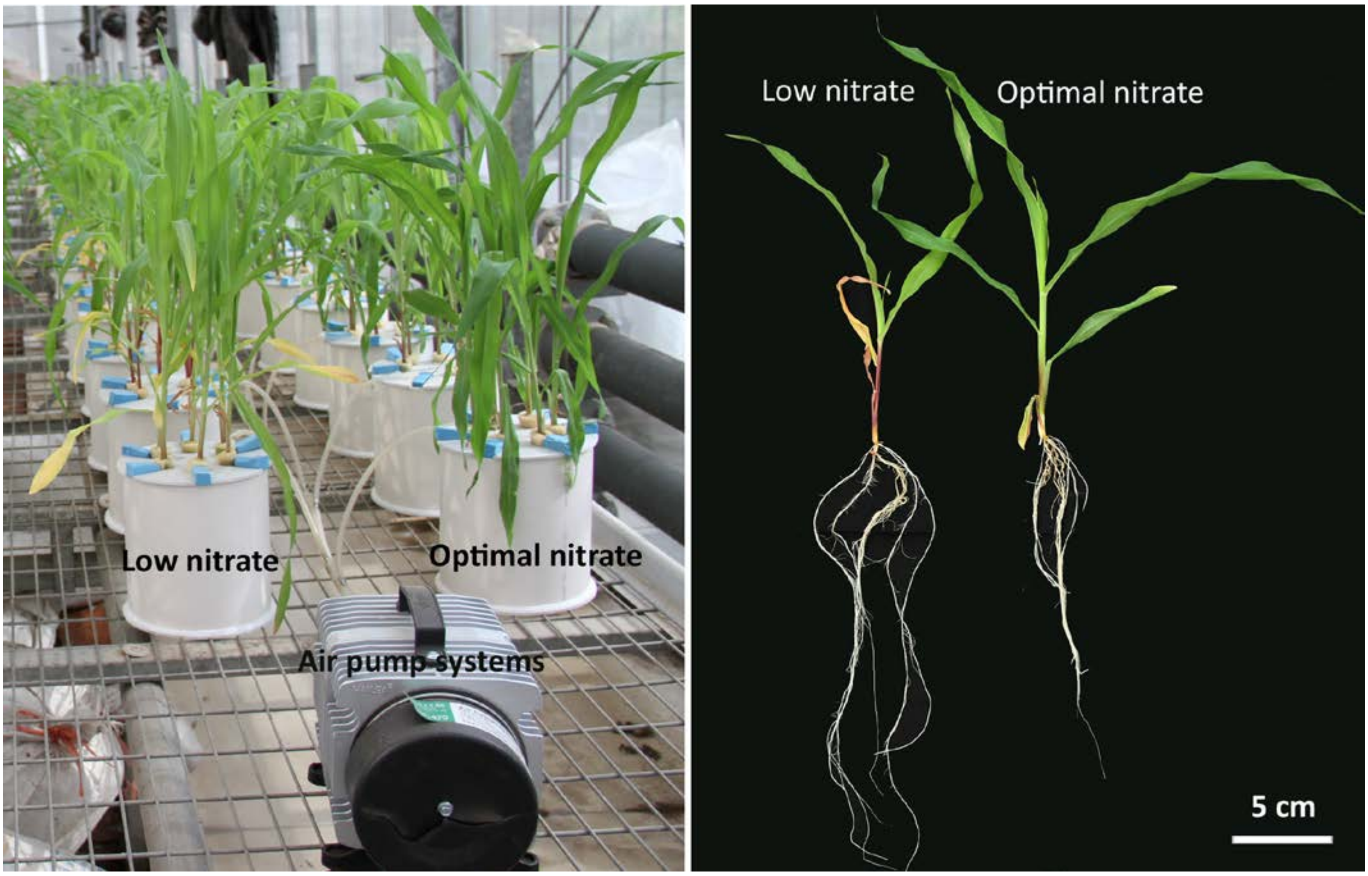


TABLE S1. Statistics of small RNA and degradome sequences from maize leaves and roots in different nitrogen conditions

Generated small RNA sequence		Redundant		Unique	
		Reads	Matching B73 RefGen_v2	Reads	Matching B73 RefGen_v2
Shoots optimal N	Raw reads	11,285,708			
	High quality reads	10,913,877			
	Adaptor removed	10,763,446			
	Other non-sRNA removed (Clean reads)	9,581,621	7,883,414	2,867,972	2,048,077
	rRNA/tRNA/snRNA/snoRNA exact matches removed	7,997,767		2,747,908	
	Match known miRNAs [a]	2,094,506		978 (139/75)	
	sRNAs mapping to predicted hairpins	41,794	41,794	430	430
	Predicted hairpins sRNA abundance ≥5	41,213	41,213	411	411
Roots optimal N	Raw reads	14,846,804			
	High quality reads	14,370,615			
	Adaptor removed	14,261,256			
	Other non-sRNA removed (Clean reads)	13,690,034	9,456,325	5,377,571	3,151,133
	rRNA/tRNA/snRNA/snoRNA exact matches removed	11,924,606		5,144,570	
	Match known miRNAs [a]	1,983,157		904 (135/90)	
	sRNAs mapping to predicted hairpins	20,402	20,402	390	390
	Predicted hairpins sRNA abundance ≥5	20,117	20,117	354	354
Shoots low N	Raw reads	13,481,331			
	High quality reads	13,094,971			
	Adaptor removed	12,927,865			
	Other non-sRNA removed (Clean reads)	11,747,649	10,089,264	3,493,347	2,548,563
	rRNA/tRNA/snRNA/snoRNA exact matches removed	9,823,295		3,362,985	

	Match known miRNAs [a]	2,608,431		950 (141/72)	
	sRNAs mapping to predicted hairpins	86,823	86,823	649	649
	Predicted hairpins sRNA abundance ≥5	85,306	85,306	595	595
Roots low N	Raw reads	13,371,488			
	High quality reads	12,948,270			
	Adaptor removed	12,836,801			
	Other non-sRNA removed (Clean reads)	11,882,191	9,383,935	4,657,891	3,103,550
	rRNA/tRNA/snRNA/snoRNA exact matches removed	10,274,213		4,491,085	
	Match known miRNAs [a]	2,082,051		953 (138/80)	
	sRNAs mapping to predicted hairpins	18,535	18,535	408	408
	Predicted hairpins sRNA abundance ≥5	18,241	18,241	385	385

Generated degradome sequence		Redundant		Unique	
		Reads	Matching B73 RefGen_v2	Reads	Matching B73 RefGen_v2
Shoots optimal N	Raw reads	32,561,734			
	High quality reads	32,154,730			
	Adaptor removed	32,001,820			
	Other non-sRNA removed (Clean reads)	30,486,153	25,841,152	2,900,782	1,745,716
	rRNA/tRNA/snRNA/snoRNA exact matches removed	29,480,132		2,889,738	
	PolyN removed	29,457,989		2,886,347	
	Degraded mRNA associate with miRNAs	27,664 (75) [b]			
Roots optimal N	Raw reads	24,124,886			
	High quality reads	23,675,597			
	Adaptor removed	23,582,687			
	Other non-sRNA removed (Clean reads)	20,777,510	14,250,960	3,194,074	1,723,813
	rRNA/tRNA/snRNA/snoRNA exact matches removed	20,056,495		3,180,523	

	PolyN removed	19,852,616		3,165,658	
	Degraded mRNA associate with miRNAs	27,423 (77) [b]			
Shoots low N	Raw reads	22,090,894			
	High quality reads	21,431,091			
	Adaptor removed	21,344,431			
	Other non-sRNA removed (Clean reads)	17,420,085	15,079,203	2,598,245	1,924,756
	rRNA/tRNA/snRNA/snoRNA exact matches removed	16,967,442		2,587,257	
	PolyN removed	16,951,151		2,583,637	
	Degraded mRNA associate with miRNAs	29,699 (91) [b]			
Roots low N	Raw reads	27,034,971			
	High quality reads	26,551,921			
	Adaptor removed	26,411,771			
	Other non-sRNA removed (Clean reads)	23,114,461	18,813,556	4,017,159	2,633,011
	rRNA/tRNA/snRNA/snoRNA exact matches removed	22,640,904		4,003,159	
	PolyN removed	22,601,132		3,990,300	
	Degraded mRNA associate with miRNAs	31,147 (102) [b]			

[a] Small RNAs were matched with known mature miRNAs in miRBase 19.0. Numbers in brackets indicate the number of matching known mature miRNAs /miRNA*.

[b] Numbers in brackets indicate the number of mature miRNAs in miRBase 19.0 which were matched degraded mRNAs.