Global analysis of the sugarcane microtranscriptome reveals a unique composition of small RNAs associated with axillary bud outgrowth

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Supplementary Table S1. *Primers used in this study*

Stem-loop RT-PCR and quantitative PCR

small RNAs	*Primer*	*Primer sequence (5' -> 3')*
miRNA529	miR529_RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAGGCTG
	miR529_Foward	CCTGAGAGAAGAGAGAGAGTA
miRNA159	miR159_RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGAAGAGCT
	miR159_Foward	GCGGCGTTGGATTGAAGGG
miRNA319	miR319_RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGAGGGAGC
	miR319_Foward	GCGGCGTTGGACTGAAGGGT
miRNA160	miR160_RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTGGCAT
	miR160_Foward	CCTGAGTGCCTGGCTCCCTGT
miRNA164	miR164_RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTGCACG
	miR164_Foward	ACTGATGTGGAGAAGCAGGGCA
miRNA171	miR171_RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGAGTGA
	miR171_Foward	CCTGAGTGAGCCGAACCAATA
miRcand2	miRcand2_RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGTTCGA
	miRcand2_Foward	TCATGATGGGGGAGCAATTCG
sRNA29098	sRNA29098_RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCCTCCA
	sRNA29098_Foward	TGCTGAGTCTTATAATTTGG
Universal primer	Reverse	GTGCAGGGTCCGAGG

Supplementary Table S1. *Continued*

Gene (accession)		
SsSPL1 (TC110345)	Foward	CCCCCCAGGTGGATGTCT
	Reverse	TTGACAGAAGAGAGAGAGCACAGC
SsGAMYB (TC134732)	Foward	TGGCAGCCATGCCTTTTTA
	Reverse	GGCGATGCACACTCAGATT
SsDCL1 (TC143136)	Foward	CTGCCCATGCCAAAAGATT
	Reverse	CATCAGGCTTGTCACGGTAA
SsARF10/16 (TC141423)	Foward	GCTGTTGAATCCAATTTTCCA
	Reverse	TTGCTAAGCTGGTGGTCTGTT
SsSCL1 (TC151967)	Foward	CGAAGCTGGCAATTCCATT
	Reverse	AACGTCGAGTGGTGATGTGA
SsTCP1 (TC153622)	Foward	CACGCAGACATGAAGGAGAA
	Reverse	AAGAATTCGGGGAATCATCC
SsRibossomal 25S (TC148086)	Foward	ATAACCGCATCAGGTCTCCAAG
	Reverse	CCTCAGAGCCAATCCTTTTCC
SsGAPDH (TC77224)	Foward	TTTGAATGGCAAGCTCACTG
	Reverse	GGTGGAAACCAAATCCTCCT
SsACTIN (AY742219)	Foward	CTTAGGTTGGATCTTGCTGG
	Reverse	TTAGAAGCATTTTCGGTGGAC
SsRPL35.4 (TC57186)	Foward	CTGAAGACGGAGAGGGAAAA
	Reverse	GGCGAAGAGAAACTAACAC

* miR156 and miR444 primers have been published in Zanca *et al.* (2010).

Supplementary Table S2. *Statistics of redundant small RNA sequences from the individual libraries*

	0-DAP inactive buds	**2-DAP outgrown buds**
Total reads	6,747,965	8,300,694
High-quality reads (19 to 25 nt)	5,615,974	4,274,873
Matching Sugarcane Gene Index (%)	2,931,453 (52%)	2,170,365 (51%)
Matching Sugarcane BAC sequences (%)	796,796 (14%)	591,764 (14%)
Perfect matches to masked genomes[a]	945,479 (17%)	505,962 (12%)
Perfect matches to transcriptomes[a]	996,179 (18%)	534,008 (12%)
Other cellular small RNAs (%)		
rRNA (%)	6,377 (0.001%)	5,332 (0.001%)
tRNA (%)	6,662 (0.001%)	7,071 (0.001%)
rasiRNA (%)[b]	1,273,787 (23%)	761,824 (18%)

[a] Genome and transcriptome data from sorghum, rice, and *Arabidopsis thaliana*.

[b] sRNAs matching transposable elements or repeat sequences present in sugarcane ESTs and/or publicly available BACs.

Supplementary Table S3. *Sugarcane EST sequences similar to miniature inverted-repeat transposable elements (MITEs) and their associated small RNA (sRNA) sequences.*

No.	EST ID	evalue	MITE annotation	MITE name	sRNA ID	sRNA sequence (5`-3`)	Size (nt)	Inactive bud (0 DAP)[b]	Active Bud (2 DAP)[b]
1	TC127268	1.9e-19	*Tourist type*	*MiSc1*	7196	UUAGGCUUAAAAGAUUCGUCUCG[a]	23	1.2	7.3
					13790	AUUAGGCUUAAAAGAUUCGUCUCG[a]	24	1.6	12.2
					24967	UAGGCUUAAAAGAUUCGUCUCG[a]	22	0.5	7.3
					19629	UUAGGCUUAAAAGAUUCGUCUCGU[a]	24	1.2	4.4
2	TC141274	3.60e-13	*Tourist type*	*MiSc2*	7196	UUAGGCUUAAAAGAUUCGUCUCG[a]	23	1.2	7.3
					13790	AUUAGGCUUAAAAGAUUCGUCUCG[a]	24	1.6	12.2
					24967	UAGGCUUAAAAGAUUCGUCUCG[a]	22	0.5	7.3
					19629	UUAGGCUUAAAAGAUUCGUCUCGU[a]	24	1.2	4.4
3	TC152830	1.2e-27	*Tourist type*	*MiSc3*	7196	UUAGGCUUAAAAGAUUCGUCUCG[a]	23	1.2	7.3
					13790	AUUAGGCUUAAAAGAUUCGUCUCG[a]	24	1.6	12.2
					24967	UAGGCUUAAAAGAUUCGUCUCG[a]	22	0.5	7.3
					19629	UUAGGCUUAAAAGAUUCGUCUCGU[a]	24	1.2	4.4
4	CA282419	1.3e-28	*Tourist type*	*MiSc4*	13790	AUUAGGCUUAAAAGAUUCGUCUCG[a]	24	1.6	12.2
					24967	UAGGCUUAAAAGAUUCGUCUCG[a]	22	0.5	7.3
					7196	UUAGGCUUAAAAGAUUCGUCUCG[a]	23	1.2	7.3
					19629	UUAGGCUUAAAAGAUUCGUCUCGU[a]	24	1.2	4.4
5	CA255688	2.8e-37	*Tourist type*	*MiSc5*	7196	UUAGGCUUAAAAGAUUCGUCUCG[a]	23	1.2	7.3
					13790	AUUAGGCUUAAAAGAUUCGUCUCG[a]	24	1.6	12.2
					19629	UUAGGCUUAAAAGAUUCGUCUCGU[a]	24	1.2	4.4
					24967	UAGGCUUAAAAGAUUCGUCUCG[a]	22	0.5	7.3
6	CA129136	1.3e-05	*Tourist type*	*MiSc6*	7196	UUAGGCUUAAAAGAUUCGUCUCG[a]	23	1.2	7.3
					13790	AUUAGGCUUAAAAGAUUCGUCUCG[a]	24	1.6	12.2
					19629	UUAGGCUUAAAAGAUUCGUCUCGU[a]	24	1.2	4.4
					24967	UAGGCUUAAAAGAUUCGUCUCG[a]	22	0.5	7.3

Supplementary Table S3. *Continued*

No.	EST ID	evalue	MITE annotation	MITE name	sRNA ID	sRNA sequence (5`-3`)	Size (nt)	Inactive bud (0 DAP)[b]	Active Bud (2 DAP)[b]
7	CA168146	4.1e-18	*Pangrangja*	*MiSc7*	20347	GUCCAAAGAUUCGAUGUGACAGUU	24	3.2	2.3
8	CA238633	6.3e-20	*MITE-adh, type B*	*MiSc8*	15153	UUUGACCACUCGUCUUAUUC	20	11	0.1
					1267	CCACUCGUCUUAUUCAAAAA	20	7.7	12.4
9	CF574551	6.2e-06	*Stowaway foldback element*	*MiSc9*	29098	UCUUAUAAUUUGGAAUGGAGG	21	9.1	6.1
					14685	GUCUUAUAAUUUGGAACGGAGGGA	24	2.3	1.9
					7788	UCUUAUAAUUUGGAACGGAGGGAG	24	10.3	1.9
					27121	UUAUAAUUUGGAACGGAGGGAG	22	2.3	1.6
					4174	UCUUAUAAUUUGGGAUGGAGGG	22	2.8	0.9
					30070	UCUUAUAAUUUGGGAUGGAGG	21	5.9	4
					27767	UCUUAUAAUUUGGAACGGAGG	21	4.1	1.4

[a] small RNA sequences that perfectly match different MITE copies.

[b] Normalized expression of transcripts per million (TPM).

Supplementary Table S4. *Total reads of known and novel sugarcane microRNAs (miRNAs) candidates identified in the axillary bud libraries*

miRNA family	Sequence (5`-3`)	Size (nt)	Inactive bud (0 DAP)	Active Bud (2 DAP)	Sugarcane miRNA Precursor[a]	Alignment Positions (bases)[b]
Known						
miR156	**UUGACAGAAGAGAGUGAGCAC**[c]	**21**	**14**	**25**	**SsMIR156b/c**	**303-323**
miR159	AUUGGAUUGAAGGGAGCUCUG	21	33	43	sof-MIR159c	45-64
	CUUGGAUUGAAGGGAGCUCC	21	30	16	sof-MIR159c	199-218
	CUUGGAUUGAAGGGAGCUCUG	21	94	37	sof-MIR159c	199-217
	GUUGGAUUGAAGGGAGCUCUG	21	14	9	sof-MIR159c	200-217
	UAUGGAUUGAAGGGAGCUCUG	21	1567	552	SsMIR159	223-243
	UCUGGAUUGAAGGGAGCUCUG	21	1352	442	SsMIR159	223-243
	UGGAUUGAAGGGAGCUCUGCA	21	37	8	SsMIR159	225-245
	UGUGGAUUGAAGGGAGCUCUG	21	1317	442	SsMIR159	223-243
	UUGGAUUGAAGGGAGCUCUGA	21	32	6	SsMIR159	224-243
	UUGGAUUGAAGGGAGCUCUGC	21	999	235	SsMIR159	224-244
	UUGGAUUGAAGGGAGCUCUUU	21	41	14	SsMIR159	224-242
	UUUGAAUUGAAGGGAGCUCUG	21	62	22	SsMIR159	223-243
	UUUGCAUUGAAGGGAGCUCUG	21	31	14	SsMIR159	223-243
	UUUGGAAUGAAGGGAGCUCUG	21	23	10	SsMIR159	223-243
	UUUGGACUGAAGGGAGCUCUG	21	97	21	SsMIR159	223-243
	UUUGGAUAGAAGGGAGCUCUG	21	23	6	SsMIR159	223-243
	UUUGGAUCGAAGGGAGCUCUG	21	148	57	SsMIR159	223-243
	UUUGGAUGGAAGGGAGCUCUG	21	16	4	SsMIR159	223-243
	UUUGGAUUAAAGGGAGCUCUG	21	95	46	SsMIR159	223-243
	UUUGGAUUCAAGGGAGCUCUG	21	80	37	SsMIR159	223-243
	UUUGGAUUGAAAGGAGCUCUG	21	148	51	SsMIR159	223-243
	UUUGGAUUGAACGGAGCUCUG	21	145	57	SsMIR159	223-243
	UUUGGAUUGAAGAGAGCUCUG	21	153	48	SsMIR159	223-243
	UUUGGAUUGAAGCGAGCUCUG	21	311	110	SsMIR159	223-243
	UUUGGAUUGAAGGAAGCUCUG	21	82	35	SsMIR159	223-243
	UUUGGAUUGAAGGCAGCUCUG	21	154	50	SsMIR159	223-243
	UUUGGAUUGAAGGGAACUCUG	21	86	32	SsMIR159	223-243
	UUUGGAUUGAAGGGACCUCUG	21	291	113	SsMIR159	223-243
	UUUGGAUUGAAGGGAGAUCUG	21	21	15	SsMIR159	223-243
	UUUGGAUUGAAGGGAGCUCAG	21	27	9	SsMIR159	223-243
	UUUGGAUUGAAGGGAGCUCCG	21	138	59	SsMIR159	223-243

Supplementary Table S4.*Continued*

miRNA family	Sequence (5`-3`)	Size (nt)	Inactive bud (0 DAP)	Active Bud (2 DAP)	Sugarcane miRNA Precursor (EST)[a]	Alignment Positions (bases)[b]
	UUUGGAUUGAAGGGAGCUCGG	21	65	16	SsMIR159	223-243
	UUUGGAUUGAAGGGAGCUCUA	21	596	195	SsMIR159	223-242
	UUUGGAUUGAAGGGAGCUCUC	21	110	34	SsMIR159	223-242
	UUUGGAUUGAAGGGAGCUCUG	**21**	**550942**	**196147**	**SsMIR159**	**223-243**
	UUUGGAUUGAAGGGAGCUCUU	21	395	139	SsMIR159	223-242
	UUUGGAUUGAAGGGAGGUCUG	21	19	9	SsMIR159	223-243
	UUUGGAUUGAAGGGAGUUCUG	21	226	62	SsMIR159	223-243
	UUUGGAUUGAAGGGAUCUCUG	21	423	183	SsMIR159	223-243
	UUUGGAUUGAAGGGCGCUCUG	21	294	151	SsMIR159	223-243
	UUUGGAUUGAAGGGGGCUCUG	21	142	58	SsMIR159	223-243
	UUUGGAUUGAAGGGUGCUCUG	21	32	6	SsMIR159	223-243
	UUUGGAUUGAAGGUAGCUCUG	21	403	159	SsMIR159	223-243
	UUUGGAUUGAAGUGAGCUCUG	21	482	166	SsMIR159	223-243
	UUUGGAUUGAAUGGAGCUCUG	21	202	71	SsMIR159	223-243
	UUUGGAUUGACGGGAGCUCUG	21	147	57	SsMIR159	223-243
	UUUGGAUUGAGGGGAGCUCUG	21	93	25	SsMIR159	223-243
	UUUGGAUUGAUGGGAGCUCUG	21	42	14	SsMIR159	223-243
	UUUGGAUUGCAGGGAGCUCUG	21	51	20	SsMIR159	223-243
	UUUGGAUUGGAGGGAGCUCUG	21	100	31	SsMIR159	223-243
	UUUGGAUUGUAGGGAGCUCUG	21	30	4	SsMIR159	223-243
	UUUGGAUUUAAGGGAGCUCUG	21	154	74	SsMIR159	223-243
	UUUGGCUUGAAGGGAGCUCUG	21	121	44	SsMIR159	223-243
	UUUGGGUUGAAGGGAGCUCUG	21	117	63	SsMIR159	223-243
	UUUGGUUUGAAGGGAGCUCUG	21	44	10	SsMIR159	223-243
	UUUGUAUUGAAGGGAGCUCUG	21	94	58	SsMIR159	223-243
miR160	**UGCCUGGCUCCCUGUAUGCCA**	**21**	**177**	**974**	**n.d.**	**n.d.**
miR162	UCGAUAAACCUCUGCAUCCAG	21	497	503	n.d.	n.d.
miR164	**UGGAGAAGCAGGGCACGUGCA**	**21**	**34**	**148**	**n.d.**	**n.d.**
miR166	UAGGACCAGGCUUCAUUCCCC	21	12	16	CN607727	178-196
	UCGGACCAGGCCUCAUUCCCC	21	16	19	CN607727	176-196
	UCGGACCAGGCUUCAAUCCCU	21	1044	2141	CN607727	176-195
	UCGGACCAGGCUUCAUCCCCC	21	9	15	CN607727	176-196

Supplementary Table S4.*Continued*

miRNA family	Sequence (5`-3`)	Size (nt)	Inactive bud (0 DAP)	Active Bud (2 DAP)	Sugarcane miRNA Precursor (EST)[a]	Alignment Positions (bases)[b]
	UCGGACCAGGCUUCAUUCCUC	21	275	956	CN607727	176-194
	UCGGACCAGGCUUCCUUCCCC	21	5	26	CN607727	176-196
	UCUCGGACCAGGCUUCAUUCC	21	158	88	CN607727	174-194
	UUUCGGACCAGGCUUCAUUCC	21	15	8	CN607727	174-194
miR167	UGAAGCUGCCAGCAUGAUCUA	21	24	14	SsMIR167	3-22
	UGAAGCUGCCAGCAUGAUCUG	21	20	35	SsMIR167	3-23
miR168	UAGCUUGGUGCAGAUCGGGAC	21	30	65	SsMIR168	18-38
	UCGCUUGGCGCAGAUCGGGAC	21	10	17	SsMIR168	18-38
	UCGCUUGGGGCAGAUCGGGAC	21	14	31	SsMIR168	18-38
	UCGCUUGGUGCAGAUCGGGAC	21	6424	16661	SsMIR168	18-38
	UGGCUUGGUGCAGAUCGGGAC	21	9	33	SsMIR168	18-38
	UUGCUUGGUGCAGAUCGGGAC	21	11	23	SsMIR168	18-38
miR169	CAGCCAAGGAUGACUUGCCGG	21	37	16	SsMIR169	3-22
miR171	**UGAGCCGAACCAAUAUCACUC**	**21**	**41**	**510**	**n.d.**	**n.d.**
	UGAGCCGAAUCAAUAUCACUC	21	1	71	n.d.	n.d.
	UGAUUGAGCCGUGCCAAUAUC	21	26	565	n.d.	n.d.
	UUGAGCCGUGCCAAUAUCACG	21	58	781	n.d.	n.d.
miR172	AGAAUCUUGAUGAUGCUGCAU	21	7	14	n.d.	n.d.
miR319	UUGGACUGAAGGGCGCUCCCU	21	28	3	SsMIR319	15-35
	UUGGACUGAAGGGGGCUCCCU	21	27	7	SsMIR319	15-35
	UUGGACUGAAGGGUGCUCCCU	**21**	**14457**	**3268**	**SsMIR319**	**15-35**
miR393	UCCAAAGGGAUCGCAUUGAUC	21	44	20	n.d.	n.d.
miR394	UUGGCAUUCUGUCCACCUCC	20	49	60	n.d.	n.d.
miR395	UGAAGUGUUUGGGGGAACUC	20	17	32	n.d.	n.d.
miR396	UCCACAGGCUUUCUUGAACUG	21	482	302	SsMIR396	8-21
	UUCCACAGCUUUCUUGAACUG	21	29	18	SsMIR396	1-21
miR397	UCAUUGAGUGCAGCGUUGAUG	21	0	29	n.d.	n.d.
miR398	UGUGUUCUCAGGUCGCCCCCG	21	0	23	n.d.	n.d.
miR399	UGCCAAAGGAGAGUUGCCCUG	21	31	7	n.d.	n.d.
miR408	CUGCACUGCCUCUUCCCUGGC	21	5	39	SsMIR408	158-178
miR444	**UGCAGUUGUUGCCUCAAGCUU**	**21**	**3**	**29**	**SsMIR444a**	**84-104**
miR444b.2	UGCAGUUGUUGUCUCAAGCUU	21	5	15	SsMIR444b	82-102

Supplementary Table S4.*Continued*

miRNA family	Sequence (5`-3`)	Size (nt)	Inactive bud (0 DAP)	Active Bud (2 DAP)	Sugarcane miRNA Precursor (EST)[a]	Alignment Positions(bases)[b]
miR444d.3	UUGUGGCUUUCUUGCAAGUUG	21	13	29	ssp-MIR444b	64-84
miR529	**AGAAGAGAGAGAGUACAGCCU**	**21**	**61**	**88**	**n.d.**	**n.d.**
	GAAGAGAGAGAGUACAGCCU	20	41	0	n.d.	n.d.
miR827	UUAGAUGACCAUCAGCAAACA	21	121	373	SsMIR827	106-126
	UUAGAUGACCAUCAGCGAACA	21	18	69	SsMIR827	106-126
	UUAGAUGGCCAUCAGCAAACA	21	16	201	SsMIR827	106-126
miR1878	ACUUAGUCUGGAUACUAUAAAAGA	24	224	44	n.d.	n.d.
Novel miRNA candidates						
miRcand1	CCTTGTCTTATCCTCCCTCGA	21	80	3	CA257509	92-112
	CCTTGTCTTATCCTCCCTCGA	21	47	0	CA257509	92-112
	GCCTTGTCTTATCCTCCCTCG	21	45	2	CA257509	91-111
miRcand2	UGGGGAAGCAAUUCGUCGAAC	21	0	127	CA222602	167-187
	UGGGGGAGCAAUUCGUCGAAC	**21**	**165**	**2141**	**CA222602**	**167-187**

[a] Sugarcane miRNA precursors described in Zanca *et al.* (2010) and/or identified in this work with sRNA matches.

[b] Position in the miRNA precursor sequence in which a particular sRNA is aligned, when applicable.

[c] miRNA sequences in **bold** were used as templates for the stem-loop RT-qPCR primers.

n.d. Not determined.

Supplementary Table S5. *Predicted target sequences of known and novel sugarcane microRNAs (miRNAs) identified in the axillary bud libraries*

miRNA family	**Target gene**	**Score**^{a}	**Putative function**
Known miRNAs			
miR156	Described in Zanca *et al*. 2010	1	Squamosa promoter binding protein-like proteins (SPLs)
miR159	Described in Zanca *et al*. 2010	2	GAMyb-like protein
miR160	TC149461, CF574323, TC141423	1	ARF protein
miR162	n.d.[b]	n.d.	n.d.
miR164	CA293713, CA274870, CA285948	1	NAC family transcription factor
miR166	CA226194,TC99735,TC93578, TC85112	2	Class III HD-Zip protein
miR167	Described in Zanca *et al*. 2010	2, 3	Auxin response factor
miR168	Described in Zanca *et al*. 2010	3	ARGONAUTE1 (AGO1)-like protein
miR169	Described in Zanca *et al*. 2010	3	CCAAT-box transcription factor complex
miR171	TC119571, TC151967	1.5	GRAS family transcription factor
miR172	TC99423, TC112055, TC93872, TC87233, TC81342	0.5	APETALA2-like protein
miR319	Described in Zanca *et al*. 2010	1.5	TCP family transcription factor
miR393	TC80044, TC77356, TC98041	2	Auxin signaling F-box 2 protein
miR394	TC108201, TC88841	0	F-box family protein
miR395	TC151109	0.5	ATP sulfurylase
	CA069883, TC118970	1	Putative sulfate transporter
miR396	Described in Zanca *et al*. 2010	1	Putative growth-regulating factor
miR397	TC142804, CA183151, CA170003, TC117778	0.5, 1, 3, 3	Putative laccase precursor
miR398	n.d.	n.d.	n.d.
miR399	n.d.	n.d.	n.d.
miR408	Described in Zanca *et al*. 2010	3	Blue copper-like protein
miR444	Described in Zanca *et al*. 2010	0.5, 2.5	Putative MIKC-type MADS-box transcription factors
miR444b.2	Described in Zanca *et al*. 2010	0.5, 1.5, 3	Putative MIKC-type MADS-box transcription factors
miR444d.3	Described in Zanca *et al*. 2010	1.5	Putative MIKC-type MADS-box transcription factors
miR529	CA228340, CA072223, TC110345, TC73496	2.5, 3	Squamosa promoter binding protein-like proteins (SPLs)
miR827	Described in Zanca *et al*. 2010	2, 2.5	SPX (SYG1/Pho81/XPR1) domain-containing protein-like
	CA126628, CA251729, TC127041	3	DNA (cytosine-5)-methyltransferase 1 domain-containing protein

Supplementary Table S5. *Continued*

miRNA family	**Target gene**	**Score**[a]	**Putative function**
miR1878	TC141112	3	Homolog to Ran-Binding Protein Mog1p
Novel miRNA candidates[c]			
miRcand1	n.d.	n.d.	n.d.
miRcand2	CA185455, CA287982	1	PPR-like proteins

[a] Score values based on miRNA target analyzes using both pSRNATarget (Dai and Zhao, 2011) and ScmiRbase (Zanca *et al.,* 2010) servers.

[b] Not detected (n.d.) in publicly available sugarcane sequence databases.

Figure legends

Fig. S1. sRNA distribution between axillary bud libraries and sRNA counts over annotated sugarcane genomic regions. (A) Size distribution of small RNAs in 0-DAP (inactive) and 2-DAP developing buds. (B) sRNA counts over annotated genomic regions of sugarcane ancient species (BAC 51L01 from *Saccharum officinarum* and BAC 265022 from *S. spontaneum*) and the hybrid R70. Peaks on the graphs represent the total counts of the two major classes of sRNAs as described in the text. A graphical representation of the structure of the BAC regions is provided underneath each peak on the graphs. Transposons and retrotransposons are shown as small and large black diamonds, respectively, while MITEs are shown as small black arrows. Genes are represented as grey arrows.

Fig. S2. Predicted miRNA harpins identified in this study by using EST sequences and the mFOLD program.

Fig. S3. Multiple sequence alignments of sugarcane miR159 mature sequences. The alignments were done using ClustalW program and assigned to two distinct sugarcane *MIR159* precursors.

Fig. S4. Expression profiles of sugarcane reference genes evaluated through RT-qPCR in 0-, 2-, and 5-DAP developing axillary buds of sugarcane hybrid SP80-3280. All RT-qPCR experiments used inactive bud (0 DAP) as the reference sample (set to 1.0). Error bars indicate standard deviation of three biological replicates. Distinct letters indicate the significant differences at $P < 0.05$ level by Student's t-test. R.E., relative expression.

Fig. S5. Concentrations of ABA and its catabolite dihydrophaseic acid (DPA) were monitored in 0-, 2-, and 5-DAP axillary buds and expressed in nanograms per gram of dry weight (ng/g DW). Error bars indicate standard deviation of three replicates.

Fig. S1

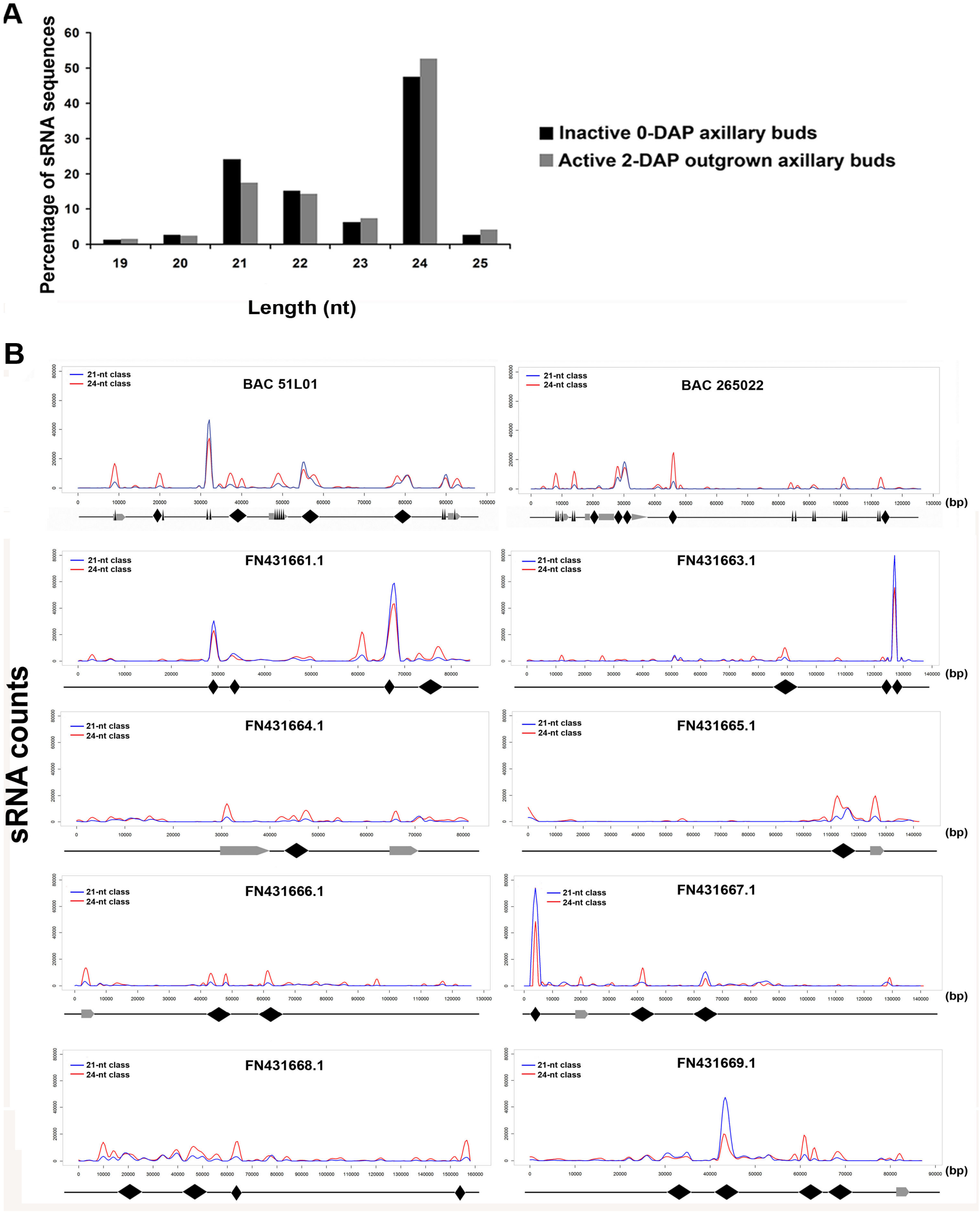

A
Percentage of sRNA sequences
0
10
20
30
40
50
60
19
20
21
22
23
24
25
Length (nt)
Inactive 0-DAP axillary buds
Active 2-DAP outgrown axillary buds
B
sRNA counts
21-nt class
24-nt class
BAC 51L01
BAC 265022
FN431661.1
FN431663.1
FN431664.1
FN431665.1
FN431666.1
FN431667.1
FN431668.1
FN431669.1
(bp)

Fig. S2

SsMIRcand1
(111 bp)

```
       UCUUUGAACUA     AGUG      CU     -         GAA--  CC  A
UACCGUU           AGUCG    UCGAGG  GGAUG GAUAAGGCC     UC  CG U
GUGGUAG           UCAGU    AGCUCC  CCUAU CUGUUCCGG     AG  GC A
       -----------     GGG-      CU     U         GCAAG  CC  U
```

ΔG = -43.60

SsMIR166
(145 bp)

```
         U   UCU   U      UU          A        UUGC           -  UUCG     GG
GAAGCUAUU GCU   GAG GGAAUG  GUCUGGUUC AGGUCUCGC    GAUUUAAGGA UGA    UGCAU   \
CUUCGAUAA CGA   CUC CCUUAC  CGGACCAGG UCUAGGGUG    CUAAGUUCCU AUU    AUGUG   C
         -   ---   C      UU          C        U---           U  UUUG     UA
```

ΔG = -70.30

SsMIRcand2
(129 bp)

```
   GA  U-      C      -       U     G        C  C--   AGC   UU    C
CCG  GC  GGCCUG CGUGGG GAAGCAA UCGUC AACGGCUG AG   GCG   UUG  AGGC U
GGC  CG  CCGGAC GUACCC CUUCGUU AGCGG UUGUCGAC UC   CGC   AGC  UCCG G
   UC  UC      C      G       C     -        U  ACA   ACU   --    A
```

ΔG = -70.50

SbMIRcand2
(114 bp)

```
      UCC    --                    -  A-    A----    G   UA
GGCCUG   UGGG  AAGCAAUUCGUCGAACAGCU GC  GCGCG     GAGG UGG  G
CCGGAC   ACCC  UUCGUUAAGCGGUUUGUCGA CG  CGCGC     CUCC ACU  G
      UGU    AU                    G  CA    ACUAG    G   CC
```

ΔG = -67.70

Fig. S3

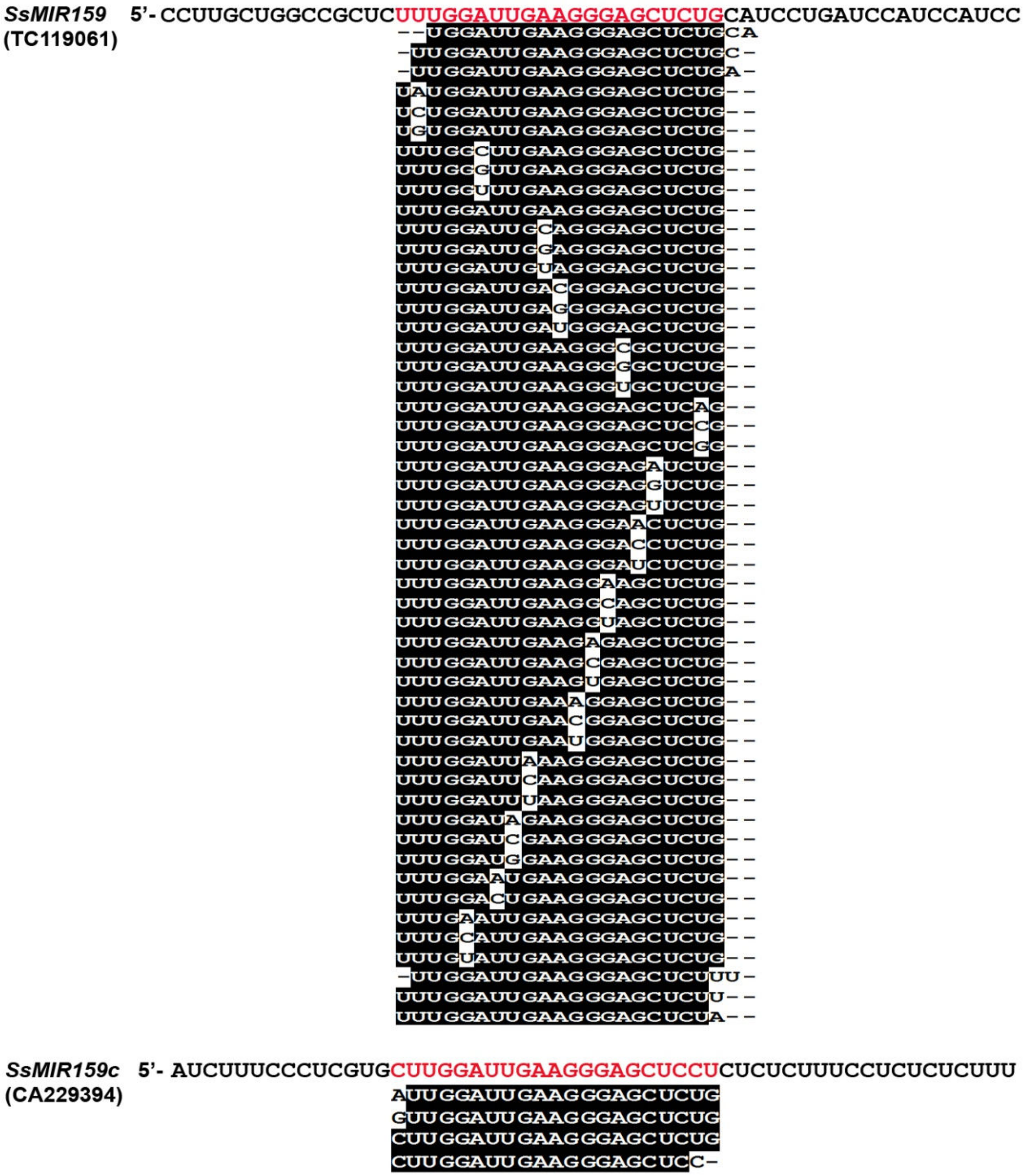

SsMIR159 5'- CCUUGCUGGCCGCUCUUUGGAUUGAAGGGAGCUCUGCAUCCUGAUCCAUCCAUCC
(TC119061)
--UGGAUUGAAGGGAGCUCUGCA
-UUGGAUUGAAGGGAGCUCUGC-
-UUGGAUUGAAGGGAGCUCUGA-
UAUGGAUUGAAGGGAGCUCUG--
UCUGGAUUGAAGGGAGCUCUG--
UGUGGAUUGAAGGGAGCUCUG--
UUUGGCUUGAAGGGAGCUCUG--
UUUGGGUUGAAGGGAGCUCUG--
UUUGGUUUGAAGGGAGCUCUG--
UUUGGAUUGAAGGGAGCUCUG--
UUUGGAUUGCAGGGAGCUCUG--
UUUGGAUUGGAGGGAGCUCUG--
UUUGGAUUGUAGGGAGCUCUG--
UUUGGAUUGACGGGAGCUCUG--
UUUGGAUUGAGGGGAGCUCUG--
UUUGGAUUGAUGGGAGCUCUG--
UUUGGAUUGAAGGGCGCUCUG--
UUUGGAUUGAAGGGGGCUCUG--
UUUGGAUUGAAGGGUGCUCUG--
UUUGGAUUGAAGGGAGCUCAG--
UUUGGAUUGAAGGGAGCUCCG--
UUUGGAUUGAAGGGAGCUCGG--
UUUGGAUUGAAGGGAGAUCUG--
UUUGGAUUGAAGGGAGGUCUG--
UUUGGAUUGAAGGGAGUUCUG--
UUUGGAUUGAAGGGAACUCUG--
UUUGGAUUGAAGGGACCUCUG--
UUUGGAUUGAAGGGAUCUCUG--
UUUGGAUUGAAGGAAGCUCUG--
UUUGGAUUGAAGGCAGCUCUG--
UUUGGAUUGAAGGUAGCUCUG--
UUUGGAUUGAAGAGAGCUCUG--
UUUGGAUUGAAGCGAGCUCUG--
UUUGGAUUGAAGUGAGCUCUG--
UUUGGAUUGAAAGGAGCUCUG--
UUUGGAUUGAACGGAGCUCUG--
UUUGGAUUGAAUGGAGCUCUG--
UUUGGAUUAAAGGGAGCUCUG--
UUUGGAUUCAAGGGAGCUCUG--
UUUGGAUUUAAGGGAGCUCUG--
UUUGGAUAGAAGGGAGCUCUG--
UUUGGAUCGAAGGGAGCUCUG--
UUUGGAUGGAAGGGAGCUCUG--
UUUGGAAUGAAGGGAGCUCUG--
UUUGGACUGAAGGGAGCUCUG--
UUUGAAUUGAAGGGAGCUCUG--
UUUGCAUUGAAGGGAGCUCUG--
UUUGUAUUGAAGGGAGCUCUG--
-UUGGAUUGAAGGGAGCUCUUU-
UUUGGAUUGAAGGGAGCUCUU--
UUUGGAUUGAAGGGAGCUCUA--
SsMIR159c 5'- AUCUUUCCCUCGUGCUUGGAUUGAAGGGAGCUCCUCUCUCUUUCCUCUCUCUUU
(CA229394)
AUUGGAUUGAAGGGAGCUCUG
GUUGGAUUGAAGGGAGCUCUG
CUUGGAUUGAAGGGAGCUCUG
CUUGGAUUGAAGGGAGCUCC-

Fig. S4

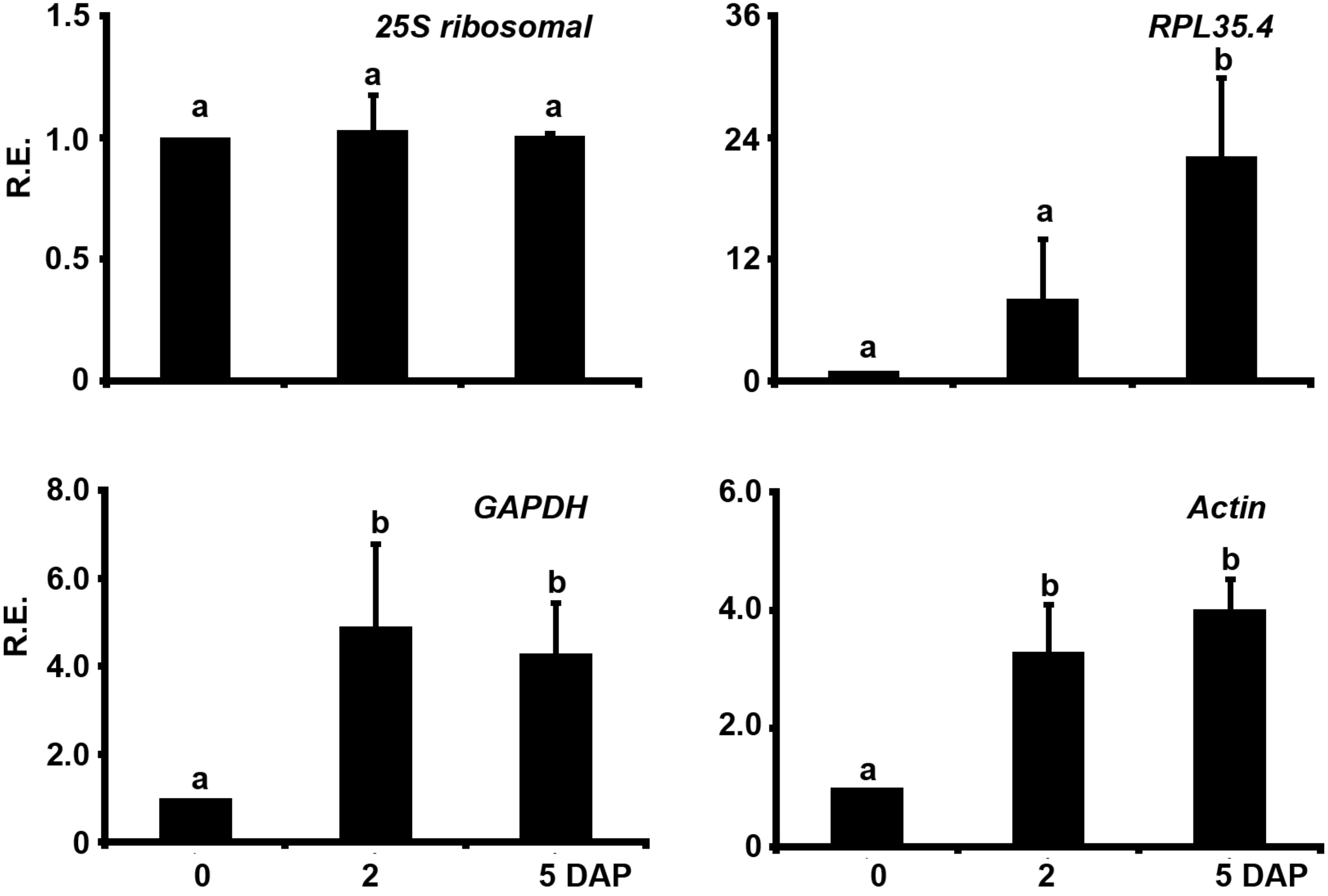
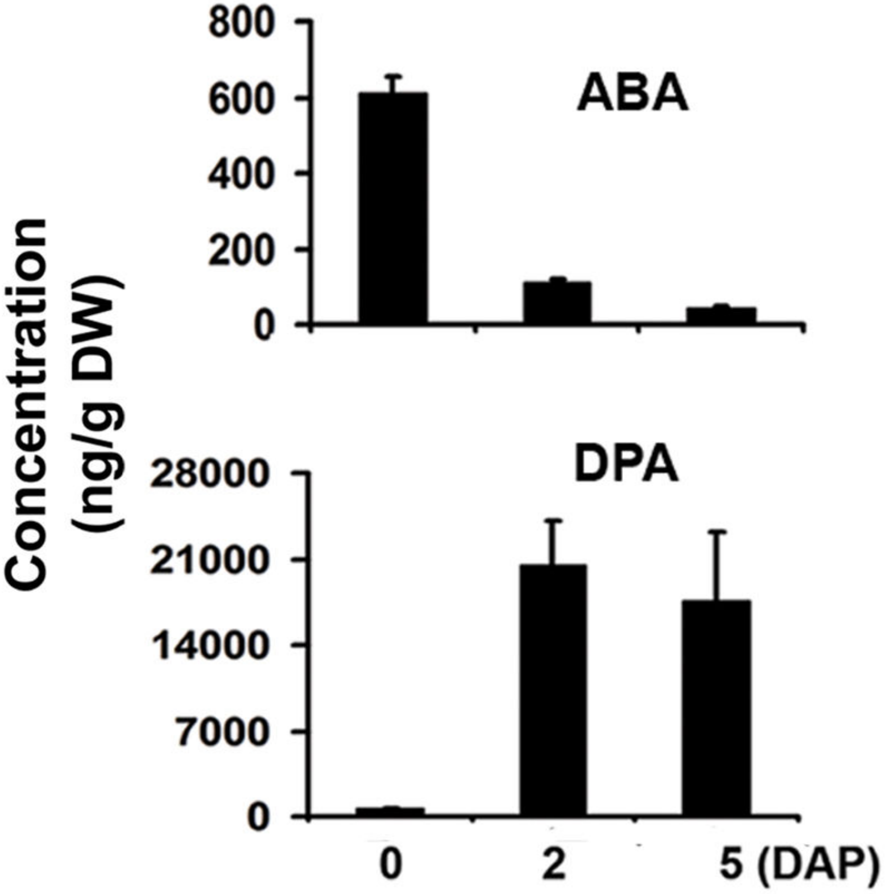

25S ribosomal
R.E.
1.5
1.0
0.5
0
a
a
a
RPL35.4
36
24
12
0
a
a
b
GAPDH
R.E.
8.0
6.0
4.0
2.0
0
a
b
b
Actin
6.0
4.0
2.0
0
a
b
b
0
2
5 DAP
0
2
5 DAP

Fig. S5



Concentration (ng/g DW)
800
600
400
200
0
ABA
DPA
28000
21000
14000
7000
0
0
2
5 (DAP)