

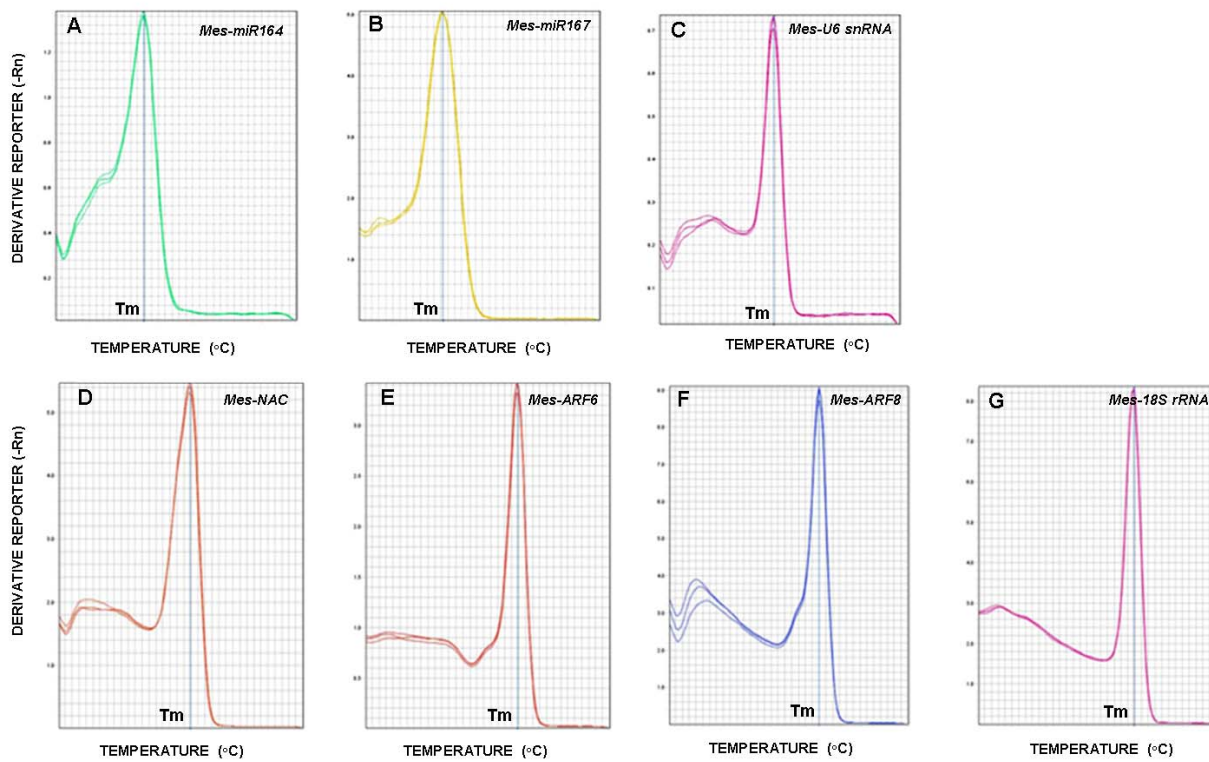


Fig. 1 Suppl. The confirmation of specificity of the primers used in RT-qPCR. Dissociation curves from the real-time PCR amplification of *mes-miR164* (A), *mes-miR167* (B), *MesU6 snRNA* (C), *MesNAC* (D), *MesARF6* (E), *MesARF8* (F), and *Mes18S rRNA* (G) are shown. The experiment was tested in a technical triplicate. In qRT-PCR, fluorescent signals from SYBR green I generally represent the amount of a target gene amplicon. Based on melting or dissociation curves, the PCR amplification of the genes of interest with selected primer pairs was observed to produce a single type of amplicon.

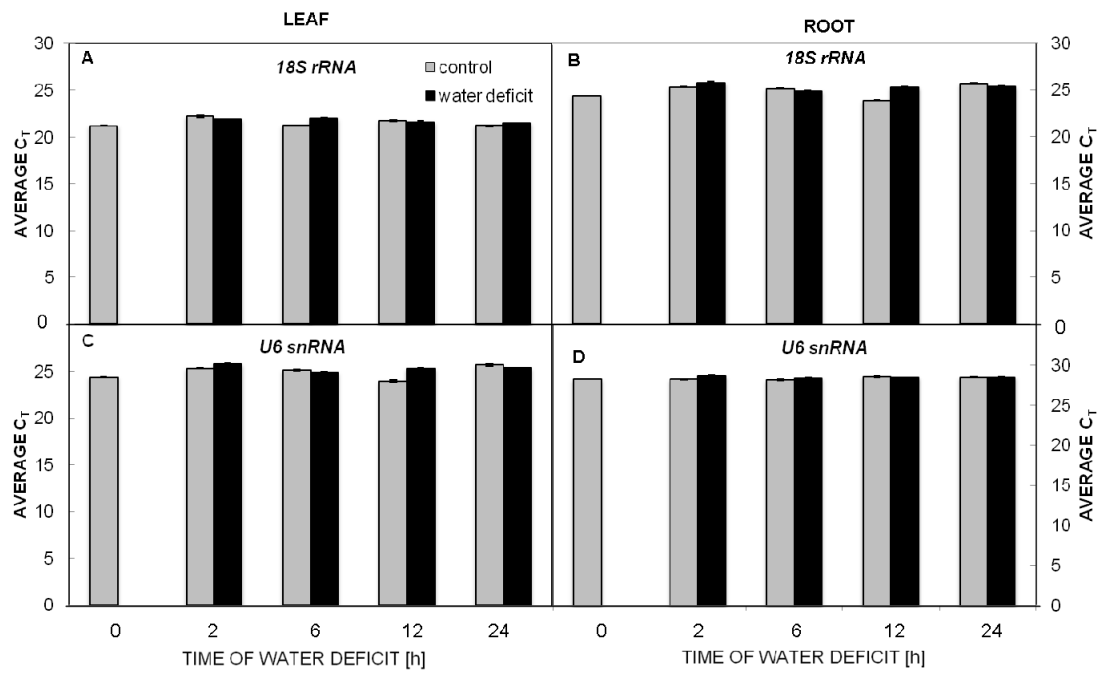


Fig. 2 Suppl. The stable expression of housekeeping genes (*18S rRNA* and *U6 snRNA*) in the leaf (A, C) and root (B, D) of cassava plantlets under the water deficit conditions. The cassava plantlets were treated with 40 % (m/v) PEG 6000 for 2, 6, 12, 24 h before sampling leaves and roots. Untreated plantlets were used as control. The *error bars* show SE, $n = 9$.

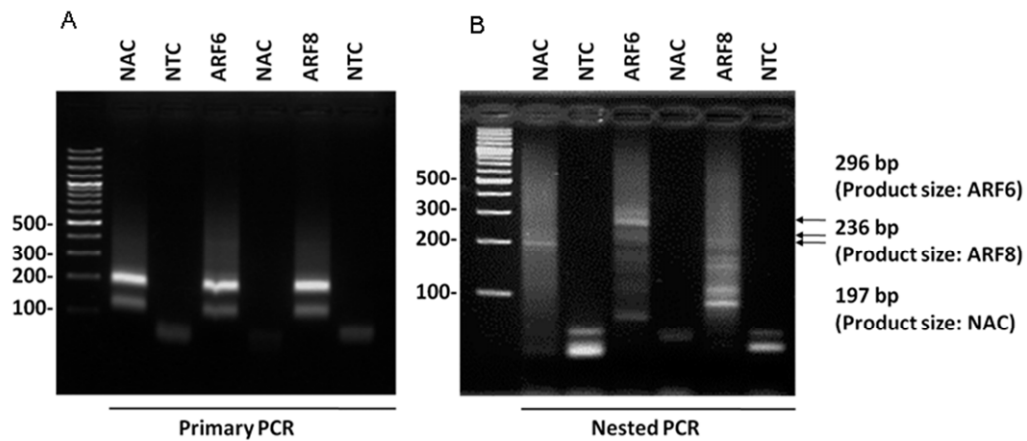


Fig. 3 Suppl. The target validation of *miR164* and *miR167* in a cassava leaf using 5'RLM-RACE. The primary (A) and nested (B) PCR amplification of *MesNAC*, *MesARF6*, and *MesARF8* in a cassava leaf. NTC - no template control, *NAC* - NAC transcription factor, *ARF6* - auxin response factor 6, *ARF8* - auxin response factor 8. The first round of PCR was observed as non-specific amplification. The nested PCR was carried out using nested gene specific primers. The specific bands of *MesNAC*, *MesARF6*, and *MesARF8* were detected at 197 bp, 296 bp and 236 bp, respectively. The amplified products from the nested PCR reactions were gel purified, ligated to a cloning vector and sequenced. According to Fig. 5, the result strongly supports the idea that *MesNAC* transcripts were the direct targets of *mes-miR164*, whereas *mes-miR167* could guide the cleavage of *MesARF8*. From this study, *MesNAC* and *MesARF8* were identified as *in vivo* targets of *mes-miR164* and *mes-miR167*, respectively, in a cassava leaf.

Table 1 Suppl. Primer sequences used for RT-qPCR analysis and for modified 5'RACE.

	Primer	Sequence 5' - 3'
RT-qPCR	miR164	AGTGTGTGTTGGAGAAGCAGGGC
	miR167	GCATCTGATGAAGCTGCCAGCA
	Universal qPCR	TGTACAGCCGGCGGAGCCGGAGATCTTA
	NAC_F	TACCGCCATTGATGGATTCT
	NAC_R	AGGGTTCGAAGAAACTGCAA
	ARF6_F	GGGGTCCTTTGGTAGGTCGC
	ARF6_R	CGGCCAGGGGTCATCACCAA
	ARF8_F	CAGGGTCGGTCGGGCGATCA
	ARF8_R	CCCCTGCTCCCCCATCTTTT
	18S rRNA_F	CGCCGGCACCTTATGAGA
	18S rRNA_R	CGTGCGGCCCAAGAACA
	U6 snRNA_F	GGGACATCCGATAAAATTGG
	U6 snRNA_R	TTTCTCGATTTGTGCGTGTC
5'RACE	GSP1 NAC	CCACTGAACATGTCCCTCTCTGTT
	GSP2 NAC	GAATGGGACAGCTTGAGCAGAGT
	GSP1 ARF6	CCACCTCTGGAAGGGTCCAAACAGA
	GSP2 ARF6	CCAAAGACCCACAGAAGTTATACCG
	GSP1 ARF8	GCGTGGAAGGAGTGGAACCTGGT
	GSP2 ARF8	GTCTCCAGCCCCATCATTACTC

Table 2 Suppl. *Cis*-regulatory elements and proportion found in miRNA promoter region.

miRNA	ABR E	AR E	AuxR E	AuxR R-core	Box- W1	C- repeat/D RE	CE3CGTC A- motif	EREGAR E- motif	HS E	LTRMB S	P- box	TAT C- box	TCA- eleme nt	TC- rich repeat	TG A	TGA- eleme nt	TGAC G- motif	WU N- motif
miR156a							2	1	1	2		1	1				1	
miR156b							1	1	1	2			2			1	1	1
miR156c		1			1		1	1	1	2	1	3	1			1	1	
miR156d	1							1	1	1	2			3		2		
miR156e	1	2		1			1		1	3			1					1
miR156f	1	1					2			2		1		2				
miR156g							1		1	1			1					
miR156h		1		1	1			1	1	3		1		2			1	1
miR156i		2							2					1				
miR156j					1									11				
miR156k	1	1	1	1		1	2		2		3		1			1		
miR159c		1					2		2		1	1	2	2				
miR159d	1							1	2				1	2				
miR160a								1	3		2					1	1	1
miR160b		1								1		1						
miR160c	1						1						1	1				
miR160e				1			1										2	
miR160f	1	1			1				1		1			4		1		
miR160g	1	1						1	2					2				
miR160h	2				1			1	2								1	
miR164b		1		1		1					1	1		2		1	2	
miR164c	2	1					2	1						2			1	
miR167a		3					5			1		2	1	3			2	
miR167b		3					5			1		2	1	3			2	
miR167c									1	1							1	
miR167e		5					3		1	1	2			1			1	
miR167f				4	1		1		1	1			1	1		1		
miR169a	1								1				6	2		1		1
miR169b	1				1						1		1					
miR169c	1			1	1		2			2			2	1		1	1	
miR169d										1				1			1	
miR169e		1						1	2				1	2				
miR169f	1	1								1				1				
miR169i								1	1		1							
miR169j					1													
miR169k		1						2	2		1			3				
miR169l					1		1		1	2	1	1	1	1				
miR169n		1					2		6		1		1	1		1	1	
miR169p	1	1						1	3		2	1		2		1	2	
miR169w	1	1						1	3		2	1		2		1	2	
miR169x	1	1						1	3		2	1		2		1	2	
miR169aa								1				1		1			1	
miR169ab	1	1					1				2	1		1			3	
miR169ac	2	1					2				1	1		1	2		3	1
miR169y							1		5					2	1			1
miR169z							1		5					2	1			1
miR171a		1			1				2	1		1		1				
miR171b	1	1					1	1	1		2	3		1		1		
miR171c		1									1			1			2	
miR171d		1												3		1	2	
miR171g								1	2					2			1	
miR171i		1					1		2	5		1	1	1				
miR171j		1							1		2			1				
miR171k		1									2			1			2	
miR172b	1	3			1						1			2			1	
miR172g											1							

[illegible]

<i>cis</i> element	No.proportion	No./miRNAs	[%]
ABRE	620.06981982	0.66666667	6.98198
ARE	720.081081081	0.77419355	8.10811
AuxRE	10.001126126	0.01075269	0.11261
AuxRR-core	170.019144144	0.1827957	1.91441
Box-W1	270.030405405	0.29032258	3.04054
C-repeat/DRE	40.004504505	0.04301075	0.45045
CE3	10.001126126	0.01075269	0.11261
CGTCA-motif	760.085585586	0.8172043	8.55856
ERE	260.029279279	0.27956989	2.92793
GARE-motif	310.03490991	0.33333333	3.49099
HSE	1450.163288288	1.55913978	16.3288
LTR	280.031531532	0.30107527	3.15315
MBS	800.09009009	0.86021505	9.00901
P-box	280.031531532	0.30107527	3.15315
TATC-box	80.009009009	0.08602151	0.9009
TCA-element	730.082207207	0.78494624	8.22072
TC-rich repeats	1080.121621622	1.16129032	12.1622
TGA box	20.002252252	0.02150538	0.22523
TGA-element	200.022522523	0.21505376	2.25225
TGACG-motif	660.074324324	0.70967742	7.43243
WUN-motif	130.01463964	0.13978495	1.46396
No. of miRNA	8881 93	9.5483871	

Table 3 Suppl. *Cis*-regulatory elements and the proportion found in the miRNA-target gene promoter region.

miRNAs	Target Phytozome	NCBI	Acc.No.	cis-element		AuxRE	AuxRR- core	Box W1
				ABRE	ARE			
miR156	SBP domain	Liguleless1 protein	TC3767	4	3			
	SBP domain	Squamosa promoter-binding protein	FF380924	3	2			
	alpha/beta hydrolase fold	sigma factor sigb regulation protein rsbq	TC11903					
miR159	unknown	myb81	30321					
miR160	Myb-like DNA-binding domain	r2r3-myb transcription factor	4606		2			
	Auxin response factor	Auxin response factor	4716					
miR164	Auxin response factor	Auxin response factor	2668	1	1			
	No apical meristem (NAM) protein	NAC domain protein	FF536822					
miR167	No apical meristem (NAM) protein	NAC domain-containing protein	26590	3				
	PAP2 superfamily	conserved hypothetical protein	DB920565; TC9480		2			
miR168	Auxin response factor	auxin response factor 6-like	1154					
	Auxin response factor	auxin response factor 8-like	1923		4			
	Translation initiation factor 2C (eIF-2C) and related proteins	Translation initiation factor 2c	28537		2			
miR169	CCAAT-binding transcription factor (CBF-B/NF-YA) subunit B	Nuclear transcription factor Y subunit A-3	TC8897	5	5			
miR171	GRAS family transcription factor	GRAS family transcription factor (GRAS42)	30763		3			
miR172	AP2 domain	AINTEGUMENTA, an APETALA2- like	6069	1				
miR319	AP2 domain	Floral homeotic protein APETALA2	5852	1				
	TCP family transcription factor	conserved hypothetical protein	DV445140		4			
miR393	F-box domain	TRANSPORT INHIBITOR RESPONSE 1 protein	TC7700					
miR395	F-BOX/LEUCINE RICH REPEAT PROTEIN	TRANSPORT INHIBITOR RESPONSE 1 protein	11998		3			
	ATP-sulfurylase (sulfate adenylyltransferase)	sulfate adenylyltransferase	TC4356		4			
miR396	Flavonol reductase/cinnamoyl- CoA reductase	cinnamoyl-CoA reductase	TC1826	2	1			
miR399	unknown	conserved hypothetical protein	28240					
miR408	-	-	-					
miR414	-	-	-					
miR477	Glutathione S-transferase, C- terminal domain	elongation factor 1 gamma	TC7923					
miR529	Elongation factor 1 gamma							
	SBP domain	LIGULELESS1 protein	TC3767	4	3			
miR530	Plus-3 domain	hypothetical protein	DB923881	1	1			
miR827	Major Facilitator Superfamily	conserved hypothetical protein	TC1622	1	3			
miR845	unknown	5-methyltetrahydrofolate:homocysteine methyltransferase	TC11724	2				
	Root hair defective 3 GTP-binding protein (RHD3)	Root hair defectiveE 3 homolog 2-like	DV449490		1			
miR1446	Short chain dehydrogenase	Short chain alcohol dehydrogenase	TC10399					
miR2111	Phospholipase/Carboxylesterase	Acyl-protein thioesterase	TC139		2			
	Kelch motif	ubiquitin-protein ligase	6028					
miR2275	Protein tyrosine kinase	G-type lectin S-receptor-like serine/threonine-protein kinase B120- like	FF534307	3	1		1	

	Stress responsive A/B Barrel domain	conserved hypothetical protein	TC1325						
total				31	47	0	1	4	

Cis-element	No.	Proportion	No./target	[%]
ABRE	31	0.102990033	0.86111111	10.299003
ARE	47	0.156146179	1.30555556	15.614618
AuxRE	0	0	0	0
AuxRR-core	1	0.003322259	0.02777778	0.3322259
Box-W1	4	0.013289037	0.11111111	1.3289037
C-repeat/DRE	1	0.003322259	0.02777778	0.3322259
CE3	0	0	0	0
CGTCA-motif	21	0.069767442	0.58333333	6.9767442
ERE	6	0.019933555	0.16666667	1.9933555
GARE-motif	17	0.056478405	0.47222222	5.6478405
HSE	46	0.15282392	1.27777778	15.282392
LTR	5	0.016611296	0.13888889	1.6611296
MBS	21	0.069767442	0.58333333	6.9767442
P-box	10	0.033222591	0.27777778	3.3222591
TATC-box	0	0	0	0
TCA-element	26	0.086378738	0.72222222	8.6378738
TC-rich repeats	31	0.102990033	0.86111111	10.299003
TGA box	2	0.006644518	0.05555556	0.6644518
TGA-element	2	0.006644518	0.05555556	0.6644518
TGACG-motif	25	0.083056478	0.69444444	8.3056478
WUN-motif	5	0.016611296	0.13888889	1.6611296
Total	301	1	8.36111111	100

No. of target's miRNAs =36