Table 1 Suppl. Primer sequences used in expression analysis of miRNAs and their target genes.

Primer	Nucleotide sequence (5'-3')	Length [nt]
miR164	AGT GTT GTT GGA GAA GCA GGG C	22
miR167	GCA TCT GAT GAA GCT GCC AGC A	22
NAC_F	TAC CGC CAT TGA TGG ATT CT	20
NAC_R	AGG GTT CGA AGA AAC TGC AA	20
ARF6_F	GGG GTC CTT TGG TAG GTC GC	20
ARF6_R	CGG CCA GGG GTC ATC ACC AA	20
ARF8_F	CAG GGT CGG TCG GGC GAT CA	20
ARF8_R	CCC CTG CTC CCC CAT CTT TT	20
U6_F	GGG ACA TCC GAT AAA ATT GG	20
U6_R	TTT CTC GAT TTG TGC GTG TC	20

Table 2 Suppl. A list of candidate miRNAs and their target genes related to root development.

miRNA	Target gene name	Target alignment	
164	*NAC1*	5'TGGAGAAGCAGGGCACGTGCA3'	*miR164*
		3'CCTAACCTCTTCGTCCCGTGCATCCGTCT5'	*NAC*
167	*ARF6, ARF8*	5'TGAAGCTGCCAGCATGATCTA3'	*miR167*
		3'TTATGTTCGACGGTCGGACTAGAGTTT5'	*ARF6*
		5'TGAAGCTGCCAGCATGATCTA3'	*miR167*
		3'TTATGTTCGACGGTCGGACTAGATACTC5'	*ARF8*

Fig. 1 Suppl. The end point reverse transcription-PCR results of miRNA-specific primers in cassava. The cDNA library from an eight-week-old storage root was used as a template. An expected PCR product size was 80 nt. M - marker, N - negative (no template).

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mes-miR164           UGGAGAAGCAGGGCACGUGCA
ath-miR164a          UGGAGAAGCAGGGCACGUGCA
ath-miR164b-5p       UGGAGAAGCAGGGCACGUGCA
osa-miR164b          UGGAGAAGCAGGGCACGUGCA
osa-miR164a          UGGAGAAGCAGGGCACGUGCA
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mes-miR167           UGAAGCUGCCAGCAUGAUCUA
ath-miR167a-5p       UGAAGCUGCCAGCAUGAUCUA
ath-miR167b          UGAAGCUGCCAGCAUGAUCUA
osa-miR167b          UGAAGCUGCCAGCAUGAUCUA
osa-miR167a-5p       UGAAGCUGCCAGCAUGAUCUA
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Fig. 2 Suppl. Multiple sequence alignment of the nucleotide sequences of *mes-miR164* and *mes-miR167* with miRNAs from other plant species; ath - *Arabidopsis thaliana*, osa - *Oryza sativa*. * indicates the positions with conserved nucleotides.

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predicted_mes-miR164c      UGGAGAAGCAGGGCACGUGCA
predicted_mes-miR164d      UGGAGAAGCAGGGCACAUGCU
predicted_mes-miR164b      UGGAGAAGCAGGGCACGUGCA
predicted_mes-miR164a      UGGAGAAGCAGGGCACGUGCA
cloned_mes-miR164          UGGAGAAGCAGGGCACGUGCA
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predicted_mes-miR167d      UGAAGCUGCCAGCAUGAUCUGA
predicted_mes-miR167e      UGAAGCUGCCAGCAUGAUCUGA
predicted_mes-miR167c      UGAAGCUGCCAGCAUGAUCUGA
predicted_mes-miR167f      UGAAGCUGCCAGCAUGAUCUU-
predicted_mes-miR167g      UGAAGCUGCCAGCAUGAUCUU-
predicted_mes-miR167a      UGAAGCUGCCAGCAUGAUCUA-
predicted_mes-miR167b      UGAAGCUGCCAGCAUGAUCUA-
cloned_mes-miR167          UGAAGCUGCCAGCAUGAUCUA-
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Fig. 3 Suppl. Multiple sequence alignment of the nucleotide sequences of cloned_*mes-miR164* and cloned_*mes-miR167* with previously predicted cassava miRNAs. * indicates the positions with conserved nucleotides.

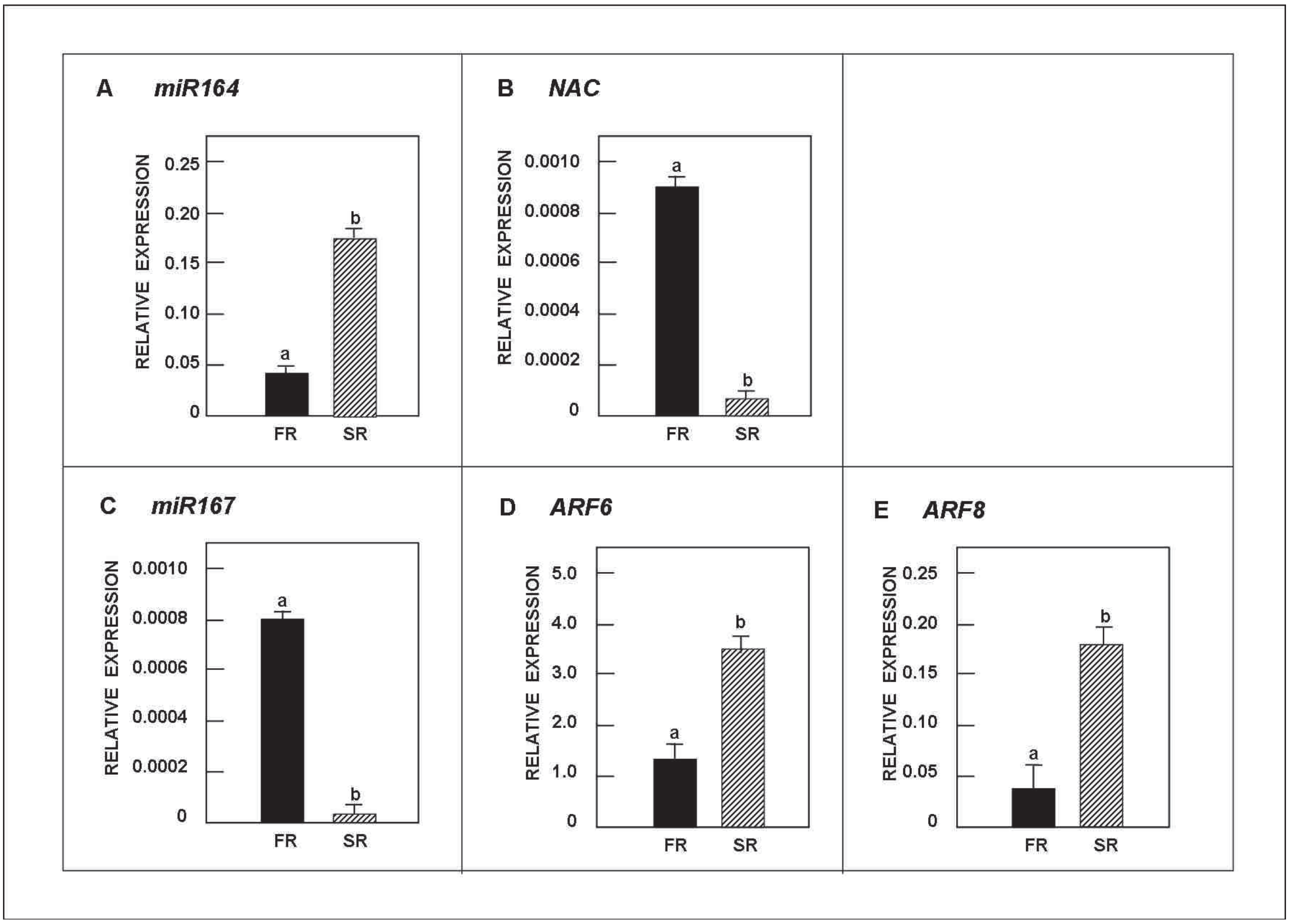

Fig. 4 Suppl. Relative expressions of miRNAs and their target genes in 12-week-old cassava plants. The expression profile of *miR164* (A), *NAC* (B), *miR167* (C), *ARF6* (D), and *ARF8* (E) were analyzed. Means ± SEs, $n = 9$, different letters indicate significant differences at $P < 0.05$. Indole acetic acid (IAA) content in fibrous roots (FR) was 1.45 ± 0.54 ng g^{-1}(f.m.) and in storage roots (SR) was 19.63 ± ng g^{-1}(f.m.).

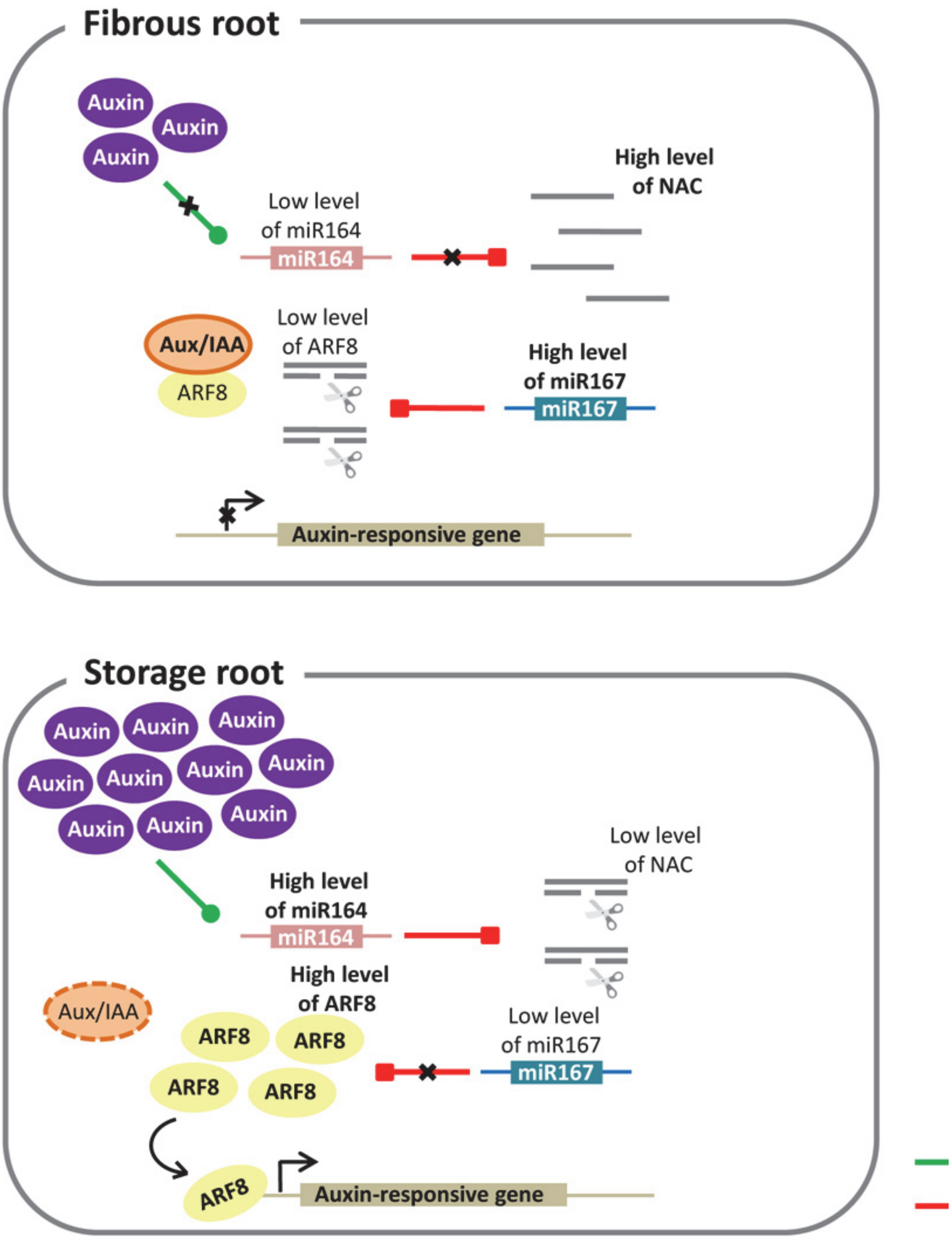

Fig. 5 Suppl. Schematic summarizing possible roles of miRNAs and their target genes on cassava storage root development. A low content of auxin in a fibrous root leads to a low transcription of *miR164*, thus allowing the full function of NAC to promote or maintain homeostasis of the fibrous root. In addition, a high amount of *miR167* causes less *ARF8* transcription, and concurrently, ARF8 interacts with AUX/IAA. The auxin-responsive genes are inactive, therefore, the formation of a fibrous root may be promoted. In a storage root, a high content of auxin activates the expression of *miR164*, which interfers with NAC transcription. The high auxin concentration degrades AUX/IAA and releases ARF8, which subsequently activates auxin-responsive genes. A low content of NAC together with the active function of auxin-responsive genes may cause storage root development.

Supplemental methods:

Quantitative determination of indole-3-acetic acid (IAA): Enzyme-linked immunosorbent assay (ELISA) was performed using an anti-IAA monoclonal competitive antibody in cassava root extracts. Fresh fibrous roots (FRs) and storage roots (SRs) (1 g) were ground with 6 cm^3 of an ice cold 80 % (v/v) methyl alcohol for 5 min. After shaking at 4 °C for 24 h and filtration, 2 cm^3 of 80 % methyl alcohol was added and after 1 h, the mixture was centrifuged at 2 000 *g* for 10 min. The collected supernatant was evaporated on a rotary evaporator to about 2 cm^3, and 1 cm^3 of petroleum ether was added into the liquid. After the liquid was layered, the layer of petroleum ether was removed, and the layer containing methyl alcohol was at the bottom. After evaporation of methyl alcohol, the aqueous solution of the sample was used for indole-3-acetic acid detection. Samples and a biotin labeled antibody were added into ELISA plate wells and washed out with phosphate-buffered saline (PBS). Then, avidin-peroxidase conjugates were added to the ELISA wells. We used 3,3',5,5'-Tetramethylbenzidine (TMB) substrate for coloring. The TMB turned firstly into blue and finally into yellow under the action of sulphuric acid. The color depth and IAA content were positively correlated.

Confirmation of miRNA precise sequences in cassava: For the validation of selected miRNA that may be involved in cassava storage root development, the end-point RT-PCR was used. A cDNA library of the miRNA from the storage root sample was amplified with a miRNA-specific primer and an adaptor primer (provided in *Ncode*™ miRNA first-strand cDNA synthesis and RT-qPCR kits, *Invitrogen*, USA). Primers for miRNA amplification are listed in Table 1 Suppl. The miRNA specific primers were designed to leave seven ‘unconstrained’ nucleotides without annealing to the 3' end of the specific target miRNA. Furthermore, ~8 nucleotides were added to the 5’ region of miRNA-specific primers in order to adjust the annealing temperature. The melting temperature (T_m) of all primers was set to be close to that of the adaptor primer. Into a 25-mm^3 PCR tube, 2 mm^3 of a cDNA template, 0.2 μM of dNTPs, 0.2 μM each of forward and reverse primers, and 2.5 units of *Taq* DNA polymerase in 1× *Taq* polymerase buffer with $MgCl_2$ was added. A reaction was carried out in a thermal cycler (*Applied Biosystems*, USA) as follows: initial denaturing at 95 °C for 3 min, 35 cycles of denaturing at 94 °C for 30 s, annealing at 60 °C for 30 s, extension at 72 °C at 30 s, and a final extension at 72 °C for 10 min. The presence of the unique miRNA-specific fragment of ~80 bp, indicated a successful amplification of the storage root cassava miRNA. Then, the amplified fragments were cloned and subsequently sequenced. After that, the nucleotide sequences were compared against registered *Arabidopsis* and rice mature miRNA sequences in *miRBase* (http://www.mirbase.org/).

Real-time quantitative PCR: The reverse transcribed cDNAs of the miRNA-enriched and target gene libraries were used as a template for real time qPCR. It was performed with an *ABI StepOnePlus* real-time PCR system (*Applied Biosystems*). The primers are listed in Table 1 Suppl. All Primers were checked for self complementarity in order to minimize non-specific amplifications. To amplify cDNA fragments, a 15-mm^3 PCR reaction mixture was prepared as follows: 2 mm^3 of a 1:10 dilution of the first strand cDNA and 0.05 μM each specific primers in 1× of *Power SYBR Green PCR Master Mix*. The PCR cycle was programmed by the *ABI 7500 system SDS Software*. After initial denaturation at 94 °C for 10 min, 40 cycles at 95 °C for 15 s and at 60 °C for 1 min for both annealing and polymerization followed. Amplification of the U6 and 18S rRNA was used as internal control for miRNA genes and target genes, respectively. For each primer pair and cDNA template, qPCR was carried out in 3 independent experiments each of which was performed in triplicate. The relative miRNA and target gene transcriptions were calculated as $2^{-\Delta Ct}$, where ΔCt represents the difference in C_T value between the reference and the target gene.

Target prediction and validation by a modified RNA ligase-mediated **5'** rapid amplification of cDNA ends **(RLM-RACE):** Because of the perfect or near perfect complementarity between a miRNA and its target mRNA, this characteristics was used for predicting plant miRNAs through homology algorithm. The target prediction was performed as previously described (Patanun *et al.* 2013). The cassava miRNAs were used to search against the cassava genome (http://www.phytozome.net/cassava). In order to predict potential cassava miRNA target genes, four selection criteria were used: *1*) mostly 4 mismatches between identified miRNA and target mRNA were allowed, *2*) no mismatches were allowed at the cleavage sites, which are positions 10 and 11, *3*) only 1 additional mismatch was allowed between positions 2 - 12 and no more than 3 mismatches between positions 12 - 25, and *4*) up to 2 consecutive mismatches were allowed. A modified RNA ligase-mediated 5’ rapid amplification of cDNA ends or 5' RLM-RACE has been predominantly used to detect an *in vivo* site-specific cleavage on target mRNAs. In order to validate target gene cleavage, a total of 250 ng of mRNA was used for ligation with RNA oligo using a *GeneRacer* kit (*Invitrogen*). In order to obtain a 5’RACE fragment, RNA oligo was ligated to the total RNA. Reverse transcription was performed using 5’ligated RNA as a template in first-strand cDNA synthesis. The first round PCR was performed using 5’RACE cDNA as a template with a 5’RACE outer primer and 5’RACE gene specific primer 1 (GSP1). The secondary nested PCR was continued using 1 mm^3 of the PCR product from the primary PCR as a template. The second round PCR were performed using 5’RACE an inner primer and 5’RACE gene specific primer 2 (GSP2). The single amplification products were subsequently cloned into the pJET1.2/blunt cloning vector and sequenced 9 different clones of *NAC* and 5 different clones of *ARF8*.