Combined Analysis of mRNA and miRNA Reveals the Banana Potassium Absorption Regulatory Network and Validation of miRNA160a

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Abstract

Potassium (K) has an important effect on the growth and development of plants. Banana contains high K content than many other fruits, and its plant requires more K nutrient in soil. However, the soil in the banana-producing areas in China is generally deficient in K. Therefore, understanding the mechanism of banana K absorption may assis in providing effective strategy to solve this problem. This study used two banana varieties with contrasting K tolerance, 'Guijiao No. 1' (low-K tolerant), and 'Brazilian banana' (low-K sensitive)to investigate K absorption mechanisms in response to low K stress through miRNA and mRNA sequencing analysis. Under low K condition, 'Guijiao No.1' showed higher plant height, dry weight, tissue K content and ATPase activity. Transcription factor analysis results showed that it was mainly concentrated in MYB, AP2-EREBP, bHLH, etc. The sequencing results showed that 'Guijiao No. 1' had 776 differentially expressed genes (DEGs) and 27 differentially expressed miRNAs (DEMs), and 'Brazilian banana' had 71 DEGs and 14 DEMs. RT-qPCR results showed that all miRNAs and mRNAs showed similar expression patterns with RNA-Seq and transcriptome. miRNA regulatory network was constructed by integrated analysis of miRNA-mRNA data. miR160a was screened out as a key miRNA, and preliminary functional validation was performed. *Arabidopsis* overexpressed miRNA160a reduced tolerance to low K, and inhibited phenotypical traits such as root length, and reduced K accumulation. The overexpressed miR160a had a targeting relationship with ARF10 and ARF16 in *Arabidopsis*. These results indicate that miRNA160a may regulate K absorption in bananas through the auxin pathway. This study provides a theoretical basis for further research on the molecular mechanism underlying the response of banana plants to low-K stress and for molecular breeding.

1. Introduction

Potassium (K) is one of the most abundant nutrients in plants, accounting for 2–10% of plant dry weight, and plays a key role in plant growth and development, such as ion homeostasis, enzyme activation, photosynthesis, stomatal movement, protein synthesis and osmotic regulation, and resistance to many biotic and abiotic stresses[10, 12, 13]. K^+ deficiency significantly inhibits the growth, root length and K uptake in banana plants [35].

MicroRNAs (miRNAs) are endogenous small non-coding RNAs, usually 20–24 nucleotides (nt) in length, which negatively regulate gene expression by promoting the degradation of target mRNA or inhibiting its translation[47]. Evidence shows that miRNAs are key factors in several plant pathways involved in most basic physiological processes of plants, including signal transduction, organ development, and response to biotic and abiotic stresses[14, 27, 29, 43].

miRNAs play a key role in regulating nutrient absorption and transportation in plants during nutrient stress. Several studies have been conducted to identify the function of miRNAs in rice, corn and wheat in response to K deficiency [44]. Under low-K stress, miR160a, miR396c and miR169h regulate plant photosynthesis[19]. These miRNAs are related to plant development, especially root development. For example, miR160, miR164, and miR390 regulate the development of lateral roots. MiR169 was inhibited

in roots treated with K deficiency and plays an important role in the K starvation response in sorghum[47]. In barley, ata-miR1432-5p can participate in the Ca^{2+} signaling pathway in response to low-K stress[40]. The miR444/MADS-box model and the pathway mediated by miR319/TCP4 and miR396/GRF may explain the difference in tolerance to low potassium between barley genotypes. The miR164c, mir169h and miR395a regulatory modules can mediate the TCA cycle, glycolysis pathway and pentose phosphate pathway under low K stress[39]. The regulatory module of miR396a-GRFs plays a negative regulatory role in *Chrysanthemum indicum* in response to salt and drought stresses. [21]. The miR156-SPL3 module regulates flowering time by regulating the expression of FT through NaKR1 under low-K^+ conditions[26]. Studies have shown that K deficiency can cause changes in the expression of ARF transcription factor genes and their corresponding miRNAs[31]. MiRNA160 acts on ARF10, which may be related to the promotion of the growth of taproot and lateral roots[16]. To date, the mechanism of miRNA-mediated low-K stress in bananas is still unclear.

In this study, we sequenced the transcriptome and miRNA of banana varieties ‘Guijiao No.1’ (low-K tolerant) and ‘Brazilian banana’ (low-K sensitive), and further analyzed the correlation between miRNA and mRNA. We constructed a key miRNA-mRNA regulatory network, from which we selected miR160a as a key miRNA, and miRNA160 acts on MaARF18. Furthermore, we constructed an overexpression vector of miRNA160a and transformed it into *Arabidopsis thaliana*. Transgenic *Arabidopsis* was subjected to low-K stress to verify the effect of miRNA160a through overexpression on low K tolerance. Our study aims to explore the mechanism of miRNA-mediated low-K stress in banana.

2. Materials And Methods

2.1 Plant materials and growth conditions

Two banana varieties ‘Guijiao No.1’ (low-K tolerant) and ‘Brazilian banana’ (low-K sensitive) were used in this study. Banana seedlings were obtained from Guangxi Plant Tissue Culture Co., Ltd., China. Seedlings were cultivated in a hydroponic experiment conducted in a greenhouse at Huaqiao University, China. The hydroponic system included ia plastic hydroponic tank filled with nutrient solution and connected to a ventilation device. Modified Hoagland nutrient solution (1/2 strength) was used, except K, which was adjusted as 0.025 mmol L^{-1} in the low K treatment and 2.5 mmol L^{-1} in the normal K treatment, using K_2SO_4. Nutrient solution was refreshed weekly.

Seedlings with similar growth were selected and pre-cultured for two weeks in a complete nutrient solution in greenhouse with a temperature of 28–35℃ in summer. A total of 60 banana seedlings were set up per genotype.

2.2 Measurement of K^+ content

Fourteen days after treatment, seedlings were harvested for assessments. The plants were divided into shoot and root.6 replicates of each setting were stored in liquid nitrogen for ATP enzyme analysis and

RNA extraction (see below), and 3 replicates of each setting were cured at 105 ℃ for 15 minutes and dried at 80 ℃ for 48 hours to constant weight. After weighing for dry weights, the dried tissues were ground into powder, added concentrated HNO_3 and digested for 4h using Multiwave PRO (Anton Paar) the determination of K concentrations using ICP-OES (Optima 7000DV).

2.3 ATP enzyme activity determination

Fresh samples of roots and shoots were wrapped in tin foil, immediately placed in liquid nitrogen, and stored at -80°C. According to the manufacturer's instructions, the H^+/K^+-ATPase activity was measured using the Plant H^+-K^+-ATPase ELISA Kit (Fankew, Shanghai, China), and the Ca^{2+}-Mg^{2+}-ATPase activity was measured using the Ca^{2+}-Mg^{2+}-ATPase activity detection kit (Solarbio, Beijing, China).

2.4 RNA Extraction

Total RNA was isolated from root samples by using RNAprep Pure plant plus kit (TIANGEN, Beijing, China) and evaluated the integrity of RNA using Agilent 2100 bioanalyzer. After passing the test, it was sent to BGI (Wuhan, China) for library construction.

2.5 Construction and sequencing of transcriptome *de novo* RNA-Seq and small RNA libraries

Total RNA was subjected to mRNA enrichment. The rRNA was removed by enriching the mRNA with polyA tails using magnetic beads with OligodT. DNA probe was used to hybridize rRNA, RNaseH selectively digested the DNA/RNA hybrid chain, and then DNA probe was digested with DNaseI, and the required RNA was obtained after purification. Fragment the obtained RNA with the interrupted buffer, reverse transcription with random N6 primer, and then the cDNA two-strand was synthesized to form double-stranded DNA. The synthetic double-stranded DNA ends were filled in and the 5'end was phosphorylated. The 3'end formed a sticky end with an "A" protruding, and then a bubbly linker with a protruding "T" on the 3'end was connected. The ligation product was amplified by PCR with specific primers. The PCR product was heat-denatured into single-stranded, and then the single-stranded DNA was circularized with a bridge primer to obtain a single-stranded circular DNA library. Sequencing was performed on the DNBSEQ platform at BGI.

For the small RNA libraries, Small RNA was enriched and purified. It was ligated with the 5-adenylated and 3-blocked adaptor to the 3' end of the small RNA fragment, and add Unique molecular identifiers (UMI) labeled Primer. 5'end adaptor was the same. Small RNA was transcribed into cDNA, and cDNA fragments was enriched by PCR amplification. Sequencing was performed on the DNBSEQ platform at BGI.

After filtering, clean tags were mapped to sRNA database such as miRBase. After sRNA annotation, those unknown tags were used to predict novel sRNA based on their architectural feature. miRA (for plants) was used to predict novel miRNA by exploring the characteristic hairpin structure of miRNA precursor. miRNA targets were predicted by TargetFinder and psRobot.

2.6 Identification and functional annotation of differentially expressed genes (DEGs) and miRNAs (DEMs)

The filter conditions of DEGs are Qvalue (Adjusted Pvalue) ≤ 0.05, |log2(fold change)|>0. The filtering conditions for DEMs are P-value ≤ 0.05,|log2foldchange|>0.5. All DEGs and target genes of DEMs are subjected to Gene Ontology and KEGG Pathway analysis. The P-value is corrected by using the Bonferroni method, a corrected P-value ≤ 0.05 was taken as a threshold. GO /KEGG terms fulfilling this condition were defined as significantly enriched GO /KEGG terms. For plant transcription factors, the ORF of Unigene was detected by getorf, and then the ORF was aligned to the transcription factor protein domain (data from TF) by using hmmsearch, and then the ability of Unigene was identified based on the characteristics of the transcription factor family described by Plant Transcriptional Factor Database.

2.7 Validation of the DEGs, DEMs and its target using RT-qPCR

To identify the accuracy and reliability of mRNA and miRNA data, RT-qPCR was used to measure the expressions of DEGs and DEMs. Total RNA used for RNA-Seq and small RNA analysis previously was reversely transcribed separately into cDNA with Stem loop primer and RT Primer Mix (Oligo dT and Random 6).RT-qPCR was performed on real-time PCR detection system (BIOER) by using SYBR® Green Pro *Taq HS* qPCR Kit (Accurate Biology, Xiamen,China). RT-qPCR reaction conditions: 30s at 95℃, followed by 40 cycles of 5s at 95℃ and 30s at 60℃. Each measurement was repeated using three biological replicates with three technical replicates for each of biological replicate. Reference gene:5SrRNA for miRNA quantification, CAC for miRNA targets quantification and ACTIN was used for the mRNA quantification. The all primers are listed in Supplementary Table S3.

2.8 Association analysis of mRNA-seq and miRNA-seq data

The Pearson correlation coefficient was calculated by the R package, which was based on the expression level of each set of target relationships in different omics in the same sample. Each product required at least 3 samples to calculate the correlation. Generally, it was considered that the absolute value of the correlation coefficient was above 0.6 to be relevant. Then the differentially expressed miRNA were combine with its targeted mRNA to construct a miRNA/mRNA regulatory network. The drawing was passed Cytoscape_v3.8.2.

2.9 Mac-miR160a overexpression vector construction and *Arabidopsis* transformation

Referred to the method of Lin et al.(2020), pCAMBIA-1300 was selected as the vector and EcorV was selected as the restriction site[17]. The recombinant expression vector pBWA(V)HS-miR160a was transformed into Agrobacterium tumefaciens strain GV3101 (Wuhan Biorun Biotechnology Co., Ltd., Wuhan, China) and then was transformed into *Arabidopsis thaliana* (Col-0) by inflorescence infection. T0

seedlings were screened in hygromycin-containing resistant medium and verified by Fragment-specific PCR and qPCR. T3 homozygous transgenic lines were used in the experiment.

2.10 Low-K^+ treatment of Transgenic *Arabidopsis*

Referred to the method of Li et al. (2017), *Arabidopsis* seeds were grown in MS medium and kept in a tissue culture room at 22°C, with a 16 h daily light period. For LK medium, KNO_3 was change to 0.05 mmol/L,KH_2PO_4 was replaced by $NH_4H_2PO_4$. Samples were collected after 14 days of LK treatment for the determination of indicators. Potassium content was determined by ICP-OES, and root length was determined by vernier caliper[15].

2.11 Statistical analysis

At least three biological replicates were evaluated for all experiments; data were presented as the mean ± standard deviation. Statistical analyses (One-Way ANOVA by LSD) were performed using the SPSS software (version 25.0). $p < 0.05$ was considered as statistically significant.

3. Results

3.1 Physiological characterizations of two banana genotypes in response to low K stress

Under low-K stress, ‘Guijiao No.1’ showed better (low-K tolerant) than ‘Brazilian banana’. The root system of ‘Guijiao No.1’ was less affected by low-K stess, and its plant height, shoot dry weight and total dry weight were also lower than those of ‘Brazilian banana’ (Fig. 1). There was a significant difference in the dry weight of roots ($P < 0.05$), Brazilian banana was decreased by 54.39% while ‘Guijiao No.1’ was only decreased by 14.73% (Fig. 1e); at the same time, the shoot potassium content of ‘Guijiao No.1’ was decreased by 34.06%, while that of ‘Brazilian banana’ was decreased by 49.39%, and the aboveground potassium accumulation of ‘Guijiao No.1’ and ‘Brazilian banana’ was decreased by 33.95% and 56.36%, respectively (Fig. 2); under low K^+ conditions, the shoot H^+/K^+-ATPase and Ca^{2+}/Mg^{2+} -ATPase of ‘Guijiao No.1’ was increased significantly ($P < 0.05$), the root Ca^{2+}/Mg^{2+}-ATPase of ‘Guijiao No.1’ also was increased significantly ($P < 0.05$) (Fig S1).

3.2 Overview of RNA-Seq and small RNA sequencing

The DNBSEQ platform was used for RNA sequencing, and produced an average of 6.39 Gb clean read. The average comparison rate of the sample comparison genome was 80.90%, and the average comparison rate of the comparison gene set was 77.30%; the 1,035 new genes were predicted; the total number of detected expressed genes was 30652, of which the known genes were 29,623 (Table S1). The gene expression distribution map showed that most of the genes were distributed in FPKM1-10 and FPKM ≥ 10 (Fig. 3a). The results of transcription factors showed that they were mainly enriched in MYB

(abiotic stress response) and AP2- EREBP (plant development, regulation miRNA, potassium related), bHLH (abiotic stress response), NAC and WRKY (potassium related) (Fig. 3c).

In order to understand the response of miRNA to low K stress, we constructed 12 sRNA libraries for SE50 sequencing. The 12 databases produced an average of 28.96 million raw reads, and an average of 27.00 million clean reads were obtained after removing low-quality reads, and the average percentage of clean read was 93.23%. The average comparison rate of the sample to the genome was 72.2%. The ratio of base quality > 20 in clean reads were 98.9% (Table S2).The length distribution displayed the 21nt and 24nt small RNA were the most abundant type (Fig. 3b).

3.3 Analysis of differentially expressed genes(DEGs)

In order to identify differentially expressed genes (DEGs) under low K stress, DEseq2 software was used to set Qvalue (Adjusted Pvalue, q) ≤ 0.05 as the screening condition. Among them, 71 significantly DEGs were screened in 'Brazilian banana with 16 up-regulated and 55 down-regulated. 776 significantly DEGs were screened in 'Guijiao No. 1', with 447 up-regulated and 329 down-regulated (Fig. 5a). The veen diagram of DEGs showed that only 5 DEGs were co-expressed, namely 103982946, 103986668, 103991590, BGI_novel_G000350, BGI_novel_G000351 (Fig. 5b). The clustering heat map of the DEGs showed that 'Guijiao No.1' has more up-regulated genes, and 'Brazilian banana' has more down-regulated genes (Fig S2).

In order to better understand the function of DEGs, GO analysis was performed on them. 25 GO categories were identified in 'Guijiao No.1'. For biological process, cellular process(219), metabolic process(195), biological regulation(79). For cellular component, cellular anatomical entity(430), intracellular(197), protein-containing complex(40). For molecular function, catalytic activity(308), binding (298), transporter activity(46). 22 GO categories were identified in the 'Brazilian banana'. For biological process, cellular process(29), metabolic process(19), biological regulation(10). For cellular component, cellular anatomical entity(43), intracellular(19),protein-containing complex(2). For molecular function,catalytic activity(35), binding(30), transporter activity(6). KEGG pathway analysis results showed that the DEGs of 'Guijiao No. 1' were mainly enriched in phenylpropanoid biosynthesis(30), MAPK signaling pathway-plant(31), protein processing in endoplasmic reticulum(22); DEGs of 'Brazilian banana' were mainly enriched in Starch and sucrose metabolism(5), plant hormone signal transduction(5), and MAPK signaling pathway-plant (4).

3.4 Analysis of differentially expressed miRNAs(DEMs)

In order to identify miRNAs related to low K^+ stress, log2foldchange>|0.5|, $p < 0.05$ was used as conditions to screen out significantly different miRNAs(DEMs). 14 and 27 DEMs were identified in 'Brazilian banana ' and 'Guijiao No.1' respectively (Fig. 6a). The expression heat map of DEMs showed that 'Guijiao No.1'had more down-regulated miRNAs than 'Brazilian banana ' (Fig. 6b).

In order to understand functional analysis of miRNAs predicted targets, we performed GO and KEGG pathway analysis on the target genes of DEMs. The GO results showed that the target genes of 'Brazilian

banana' and 'Guijiao No. 1' DEMs were both related to binding, cell, cell part, and organelle (Fig. 7). KEGG results show that 'Brazilian banana' was mainly enriched in metabolic pathway and Tyrosine metabolism, and 'Guijiao No. 1' was mainly enriched in plant hormone signal transduction (Fig S3).

3.5 RT-qPCR validation of DEGs, DEMs

In order to identify the accuracy and reliability of RNA-Seq and small RNA data, RT-qPCR was used to measure the expressions of some DEGs and DEMs, including 16 mRNAs and 16 miRNAs randomly selected. RT-qPCR results showed that all miRNAs and mRNAs showed similar expression patterns, which indicated that the results of RNA-Seq and small RNA data were reliable(Fig. 8).

3.5 Overexpression of miRNA160a in Arabidopsis can inhibit potassium absorption

In order to identify potential miRNA-mRNA pairs related to low-K stress response, we performed expression correlation analysis on RNA-seq and miRNA data. The DEMs with log(foldchange) ≥ 1 were further screened to construct an interaction network. From the interaction network, it can be seen that 17 miRNA-mRNA pairs were produced by 8 miRNAs and 14 genes, of which 8 pairs have negative correlation effects(Fig. 9). Among the 8 negative miRNA-mRNA pairs, the expression of mi160a decreased the most, and it was differentially expressed in the two banana varieties. The target gene ARF18 was involved in the auxin response pathway, which has been shown to be involved in the regulation of plant potassium .

In order to further verify the function of miR160a, we constructed an overexpression vector and obtained *Arabidopsis* transgenic lines. We tested the expression of miR160a in transgenic lines and wild-type lines. The highest expression was TG4 which was 12.27 times that of WT, and the lowest expression TG6 was 3.24 times that of WT (Fig. 10a). We also verified the three target genes of miR160a in *Arabidopsis* and found that the expression of ARF10 and ARF17 was severely inhibited. The most severely inhibited were TG2 and TG4, which was just the opposite of the expression of miR160a (Fig. 10b, 10c). The expression of ARF16 did not have a negative correlation with miRNA160a (Fig. 10d). Overexpressed miRNA160a inhibited the growth of *Arabidopsis*, especially for root length inhibition, indicating that the tolerance of transgenic *Arabidopsis* to low-K was reduced (Fig. 11a, 11b). The potassium content of transgenic *Arabidopsis* was significantly lower than the same treatment The wild type indicates that the overexpression of miR160a inhibits the absorption of potassium (Fig. 11c).

4. Discussion

Soil potassium deficiency had become an important factor affecting crop yield and quality. Therefore, how to make bananas use potassium resources in the soil more effectively was a problem that needs to be solved urgently. According to the laboratory previous data, we screened 'Guijiao No.1' and 'Brazilian banana' as low-K tolerant and low-K sensitive genotypes, respectively. The response difference between the two genotypes of bananas was first manifested at the physiological level. The low-K tolerant variety 'Guijiao No.1' had better plant height, biomass, K concentration and accumulation under low-K stress.

Some abiotic stresses affected H^+/K^+-ATPase activity. The H^+/K^+-ATPase and Ca^{2+}/Mg^{2+} -ATPase of ‘Guijiao No. 1’ in root that showed an extremely significant increase under low-K stress. The tolerance of banana varieties under low-K^+ conditions may be related to enzyme activity Ye et al(2017)[38].

The difference in genotype response was secondly manifested at the mRNA level. ‘Guijiao No.1’ (low-K tolerant) had more DEG responses under low-K^+ conditions. The first three functions of ‘Guijiao No.1’ GO analysis mainly focus on cellular anatomical entity, catalytic activity, binding, etc. This was consistent with studies in tomatoes and Chinese yam [45, 46]. The results of KEGG showed that the first three types of pathways in ‘Guijiao No.1’ were phenylpropanoid biosynthesis (30), MAPK signaling pathway-plants (31), and protein processing in the endoplasmic reticulum (22). Phenylpropanoid compounds acted as biological signals for plants to sense external stimuli and participate in biotic and abiotic stress responses[7]. In recent years, studies in *Arabidopsis* have shown that leucine-rich repeat sequences receptor protein kinases (RLK) were at least 600, most of which were involved in the growth and development of plants[8]. The MAPK signal transduction pathway was located downstream of RLKs and played a central role in growth, development and biological and abiotic stress signaling [24]. Protein processing in the endoplasmic reticulum could confer the function of protein signal transduction[4]. DEGs in ‘Brazilian banana’ were involved in the MAPK signal pathway, plant hormone signal transduction, starch and sucrose metabolism, and other related pathways. Starch and sucrose metabolism were involved in cell wall biosynthesis, cell proliferation, cell expansion, nutrient accumulation, primary metabolism and hormone signal transduction[41].

MiRNA plays a role of post-transcriptional regulation through targeted shearing of target mRNA, and its role in regulating plant nutrient stress had been extensively studied. In this study, ‘Guijiao No.1’ showed more response differentially expressed miRNAs(DEMs). The first three GO classifications of the target genes of ‘Guijiao No.1’ and ‘Brazilian banana’ DEMs were the same, and they were binding, cell, and cell parts. The KEGG analysis of target genes showed that ‘Guijiao No. 1’ was mainly enriched in plant hormone signal transduction, while ‘Brazilian banana’ was enriched in metabolic pathways and tyrosine metabolism. Phytohormones were small molecular compounds produced in plants, but they could affect cell division, differentiation, elongation, germination and rooting, plant height, branching, flowering and fruiting, etc., and played an important regulatory role in regulating plant growth and development[33]. The tyrosine metabolic pathway was the starting point for plants to produce a variety of natural compounds with diverse structures, such as tocopherol, plastoquinone, ubiquitin, betaine, salidroside, and benzyl isoquinoline alkaloids. Among them, tyrosine metabolites, tocopherols, plastoquinone and ubiquitinone were necessary for plant survival[34].

Based on the data of mRNA and miRNA, a miRNA-mRNA regulation network was constructed. In this regulation network, 4 miRNAs and 6 target mRNAs form 8 pairs of negative regulatory effects. Four known miRNAs and 11 genes formed 14 pairs of miRNA-mRNAs in the yam tuber regulatory network[46]. Among them, novel_mir3 acted on the SPL17.The Squamosa Promoter-Binding Protein-Like (SPL) gene encoded a plant-specific transcription factor, which played a role in plant phase transition, flower and fruit development, plant configuration, gibberellin signaling, sporogenesis, and response to copper and

mycotoxins important role[5]. The feedback interaction of miR156-SPLs ran through the entire plant development process and may also exist in other plants[26]. In *Arabidopsis*, many SPL genes were regulated by miR156 after transcription, and AtSPL9 in turn positively regulated the expression of the second miRNA miR172[5]. Among them, miR160a-5p acts on ARF18. In salt-tolerant beet (*Beta vulgaris*) seedlings, miR160 acted on the target gene ARF17/ARF18 to cope with salt stress[6].MiR319_1 acted on the three target genes of GATA26, RAV1, and PCF6. GATA transcription factors were a type of transcription factors that were widely present in eukaryotes. They played an important role in the biological processes of plant light response regulation, chlorophyll synthesis, cytokinin response, and carbon and nitrogen metabolism. In addition, GATA also played an important role in plant response to stress, such as nitrogen stress, cold stress, drought stress, etc., which had been proved by research[1, 2, 42]. RAV-like proteins were a subclass of the AP2 protein family and played a key regulatory role in plant stress responses and hormone responses[37]. TCP gene family members could be divided into two major branches: TCP-P (consisting of the PCF subfamily) and TCP-C (consisting of the CIN and CYC/TB1 subfamilies)[22, 25]. The interaction between the TCP-P branch and key proteins in the hormone signal transduction pathway was involved in the regulation of hormone signals such as gibberellin, cytokinin, abscisic acid, jasmonic acid, and auxin[11]. MiR319a_1 acted on the three target genes of RAVI, PCF6 and GAMYB. Gamyb-like genes may mediated the response of GA signaling pathway during plant growth and flowering[9]. Among all the negatively regulated miRNA-mRNA pairs, the expression difference of miR160a was the most significant. Its target gene ARF was involved in the auxin pathway and may respond to low-K stress by regulating root growth. Therefore, miR160a was selected as a key miRNA for further functional validation.

In order to verify the regulatory effect of miR160a on potassium, an overexpressing miR160a vector was constructed and a miR160a overexpressed *Arabidopsis* line was obtained. Compared with WT, the TG strain showed lower dry weight, shorter root length, and lower potassium content. This indicated that overexpression of miR160a made *Arabidopsis* more intolerant to low-K stress. The study founded that miR160a acted on three target genes of atARF10, atARF16, and atARF17[20]. In our study, RT-qPCR was used to verify that the target genes of miR160a in *Arabidopsis* were atARF10 and atARF17. In *Arabidopsis*, ARF17 was involved in the auxin signal transduction pathway, affecting its embryo and root development, vegetative growth and reproductive growth[32]. miR160 degraded the target genes ARF16 and ARF10 by cutting them, regulating root tip growth and geotropism, and overexpressing miR160c in *Arabidopsis* can cause root tip development defects[18]. miR160a/b was a key regulator in potatoes, which affected the root structure of plants by cutting the mRNA of StARF10 and StARF16[36]. That miR160 was involved in the formation of adventitious roots of apple rootstocks induced by auxin[23]. miR160 directed soybean nodule development in soybeans[28]. miR160-ARF18-mediated peanut (*Arachis hypogaea* L.) response to salt stress[30]. In

Alfalfa(*Medicago Sativa* L.), overexpression of miRNA160a significantly inhibited root length. In apple (*Malus pumila* Mill.), the Mdm-miR160-MdARF17-MdHYL1 module regulated the development of adventitious roots to regulate the drought tolerance of apples[3]. In peanuts, the down-regulation of miR160a may promote the growth of primary roots and lateral roots under potassium-deficient

conditions[16]. To analyze the differences in their genotype responses, and the key difference miRNA160a was screened out by constructing a miRNA-mRNA regulatory network.miR160a had been subjected to a preliminary gene function validation. The results showed that miR160a overexpression inhibited the growth and potassium absorption of transgenic *Arabidopsis thaliana*, and played an important regulatory role in potassium absorption. We further verified the target genes ARF10, ARF17, and ARF18 of miRNA160a in transgenic *Arabidopsis thaliana* by RT-qPCR, and found that the expression of ARF10 and ARF17 was severely inhibited. This indicated that miR160a may regulate potassium absorption by regulating ARF transcription factors to participate in the auxin metabolism pathway.

In conclusion, this present study demonstrated that miR160a had an important regulatory effect on banana low-K stress. Overexpression of miR160a inhibited the growth and development of *Arabidopsis thaliana* through the target gene ARF10/17, and reduced the low-K tolerance of *Arabidopsis thaliana* by inhibiting root growth. Although the complex regulation mechanism of miR160a in low-K stress was still unclear, our results provided a possible mechanism for miR160a-ARFs module-mediated regulation of low-K stress response, and provided a new insights into the molecular mechanism of banana tolerance to low-K stress.

Declarations

Authors Contribution：W.C., T.D., J.L. and M.W. conceived the research; W.C.,C.W.and Y.T. designed and conducted the experiments; W.C., P.L. and K.C. analyzed the data; W.C. wrote the manuscript; H.Y. ,Y.C.and M.W. revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest ：The authors declare that they have no conflicts of interest.

Data availability： All relevant data can be found within the manuscript and its supporting materials

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Figures

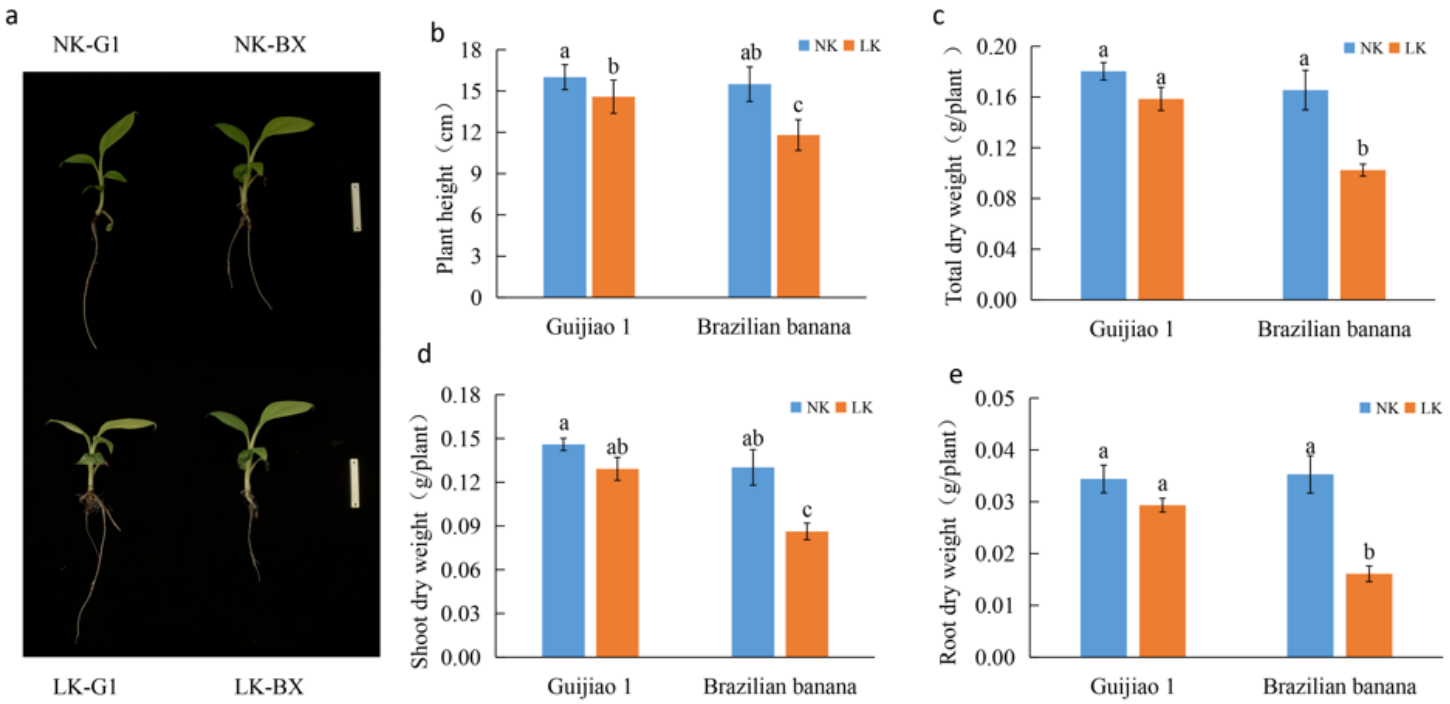

Figure 1

Response of two banana genotypes to low K stress. (a) Effects of low K stress on phenotypes (b) Effects of low K stress on plant height. (c) Effects of low K stress on total dry weight. (d) Effects of low K stress on dry weight of shoots (e). Effects of low K stress on root dry weight. NK: 2.5 mmol L^{-1} K^{+} LK:0.025 mmol L^{-1} K^{+} G1: Guijiao No.1 BX:Brazilian banana Ruler in the picture: 5 cm Symbols a, b, c, etc., indicate a signifificant difference in the *p* values of 0.05.

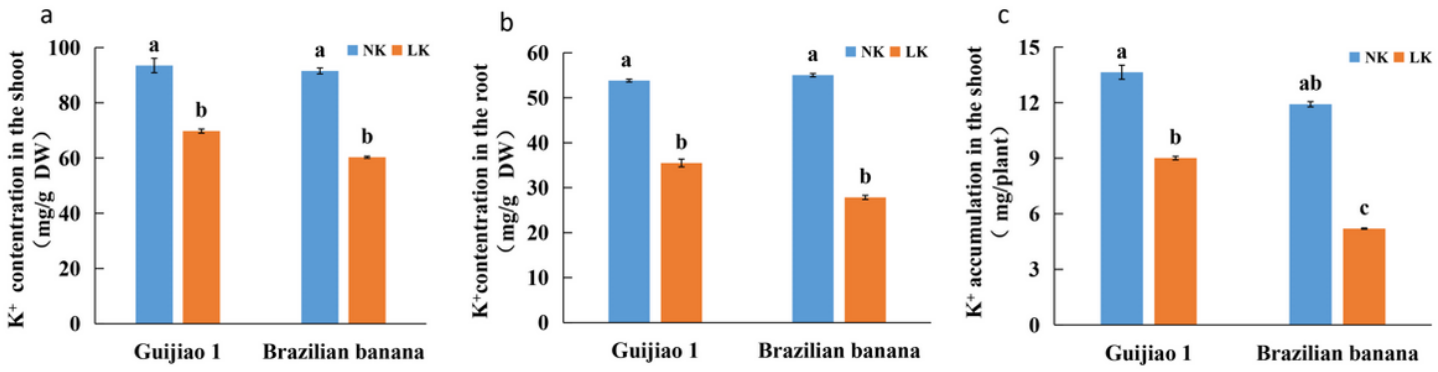

Figure 2

Effect of low K stress on potassium content. (a)K content in the shoot, (b)K content in the root, (c) K accumulation in the shoot.

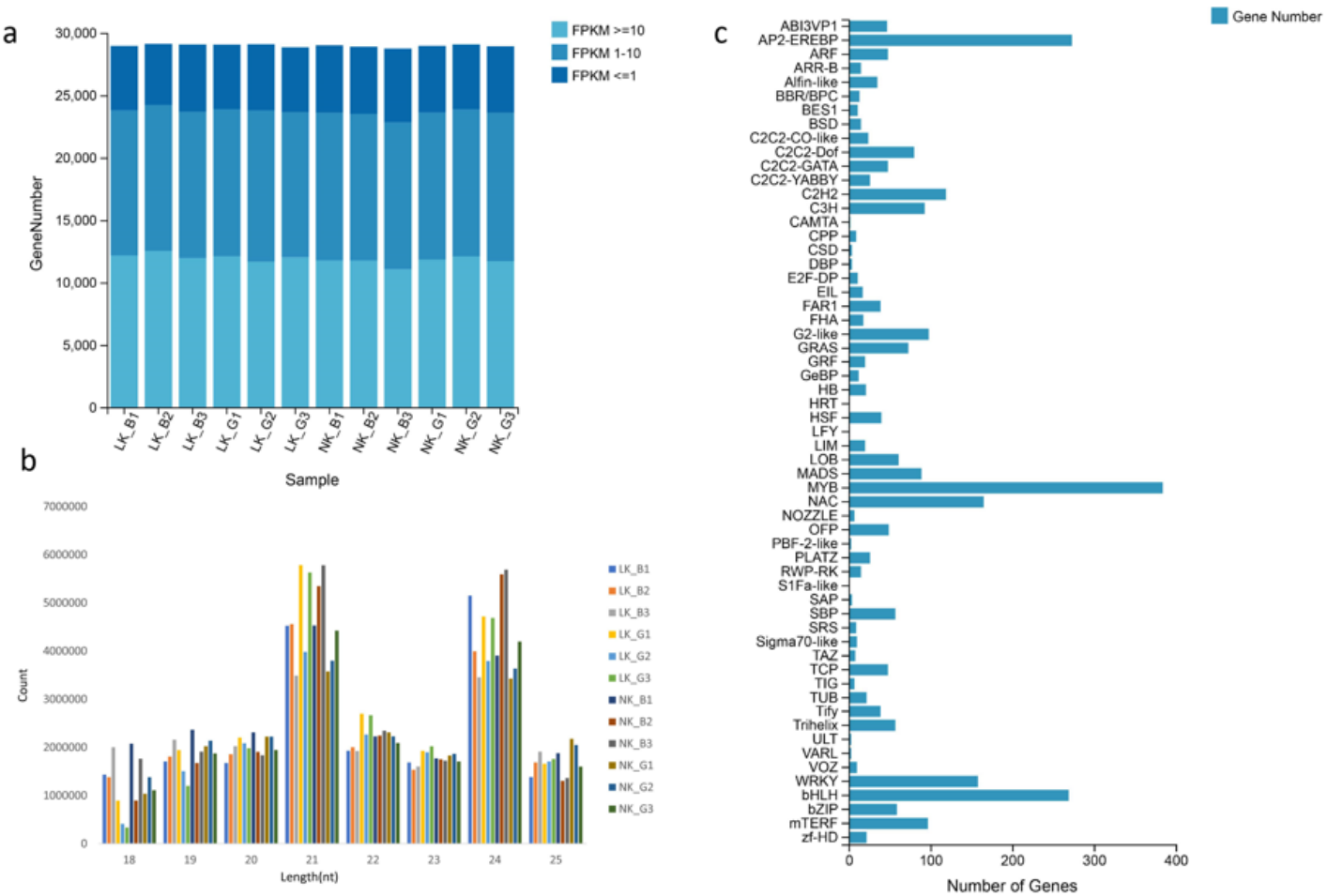

Figure 3

Basic analysis of sequencing. (a)The mRNA gene expression distribution map, (b)The sRNA length distribution map, (c) The transcription factors distribution map.

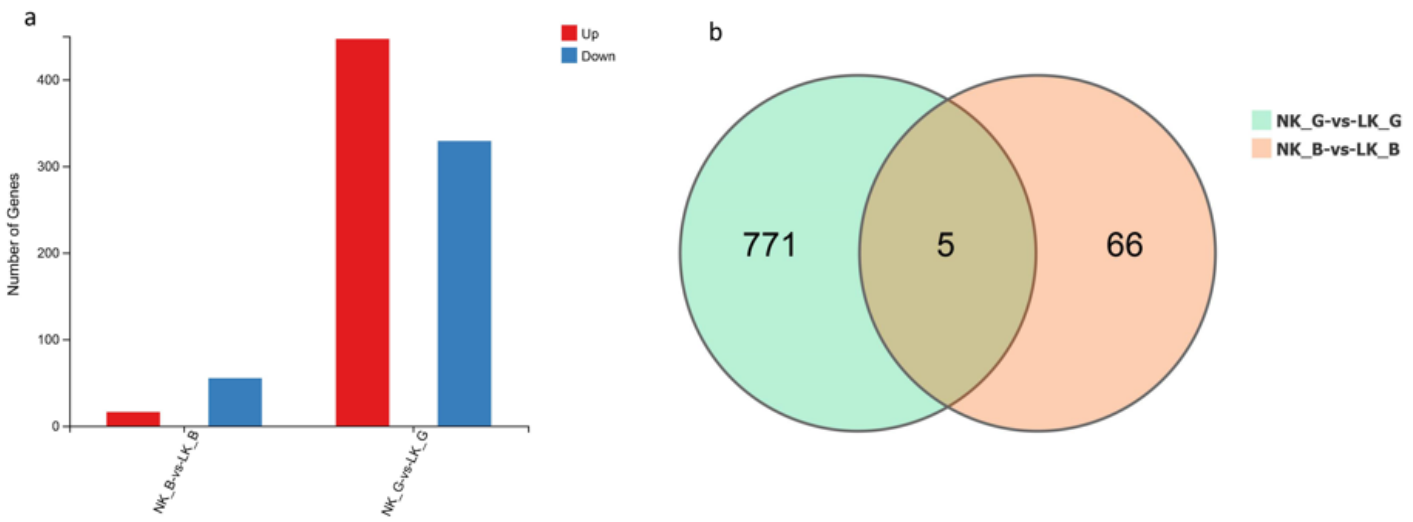

Figure 4

DEGs of Brazilian banana and Guijiao No.1 (a) number of DEGs, (b) Venn map of DEGs.

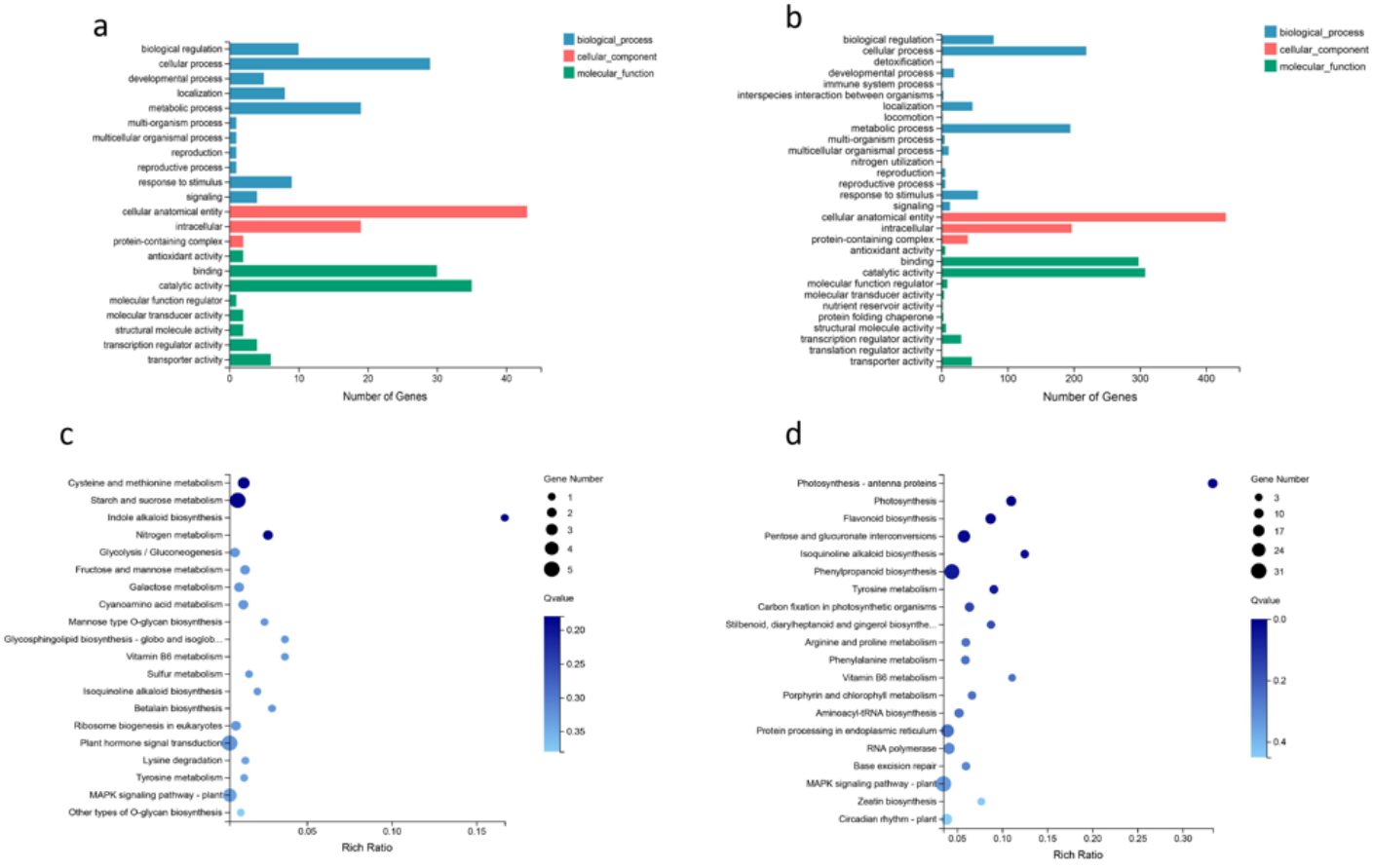

Figure 5

Gene Ontology (GO)and KEGG pathway annotation of DEGs (a)GO of Brazilian banana (b)GO of Guijiao No.1 (c)KEGG of Brazilian banana (d)KEGG of Guijiao No.1

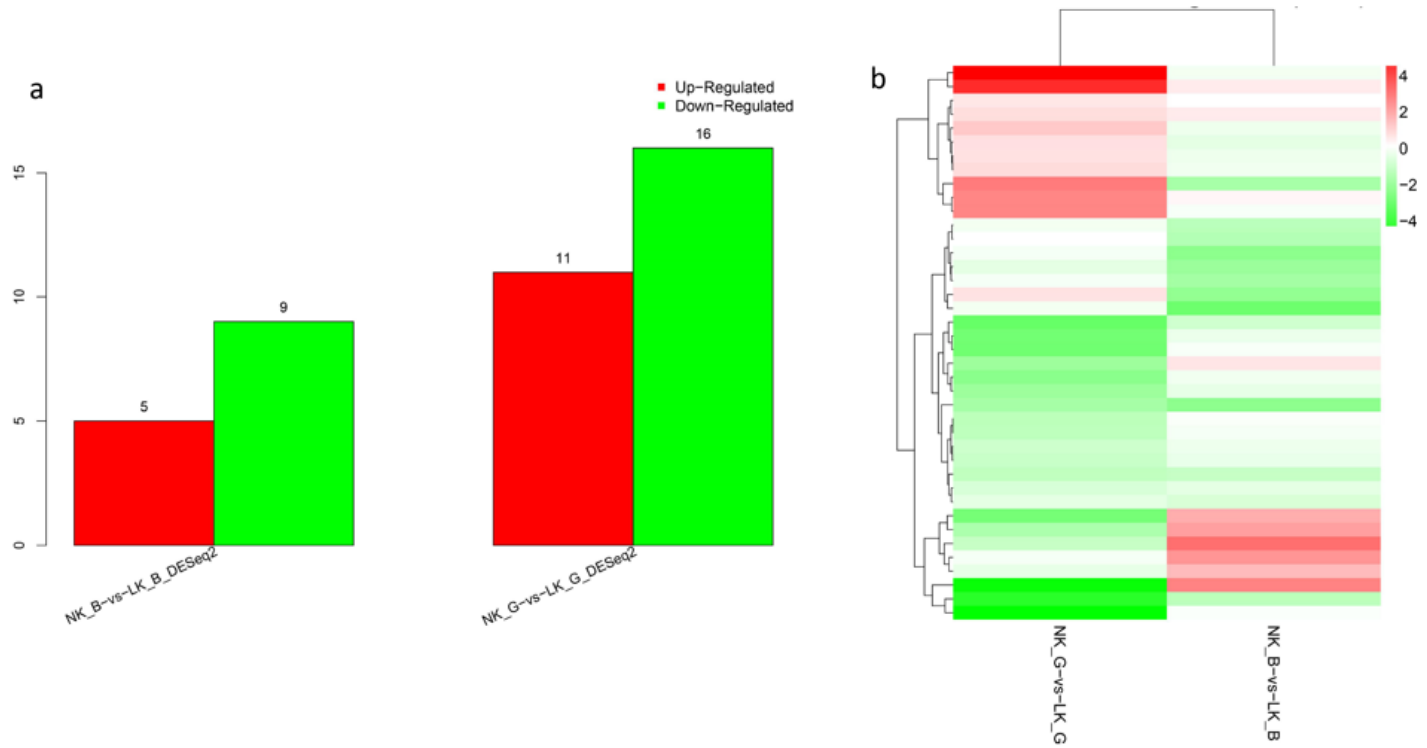

Figure 6

Number (a) and heat map (b) of differential expressed miRNAs

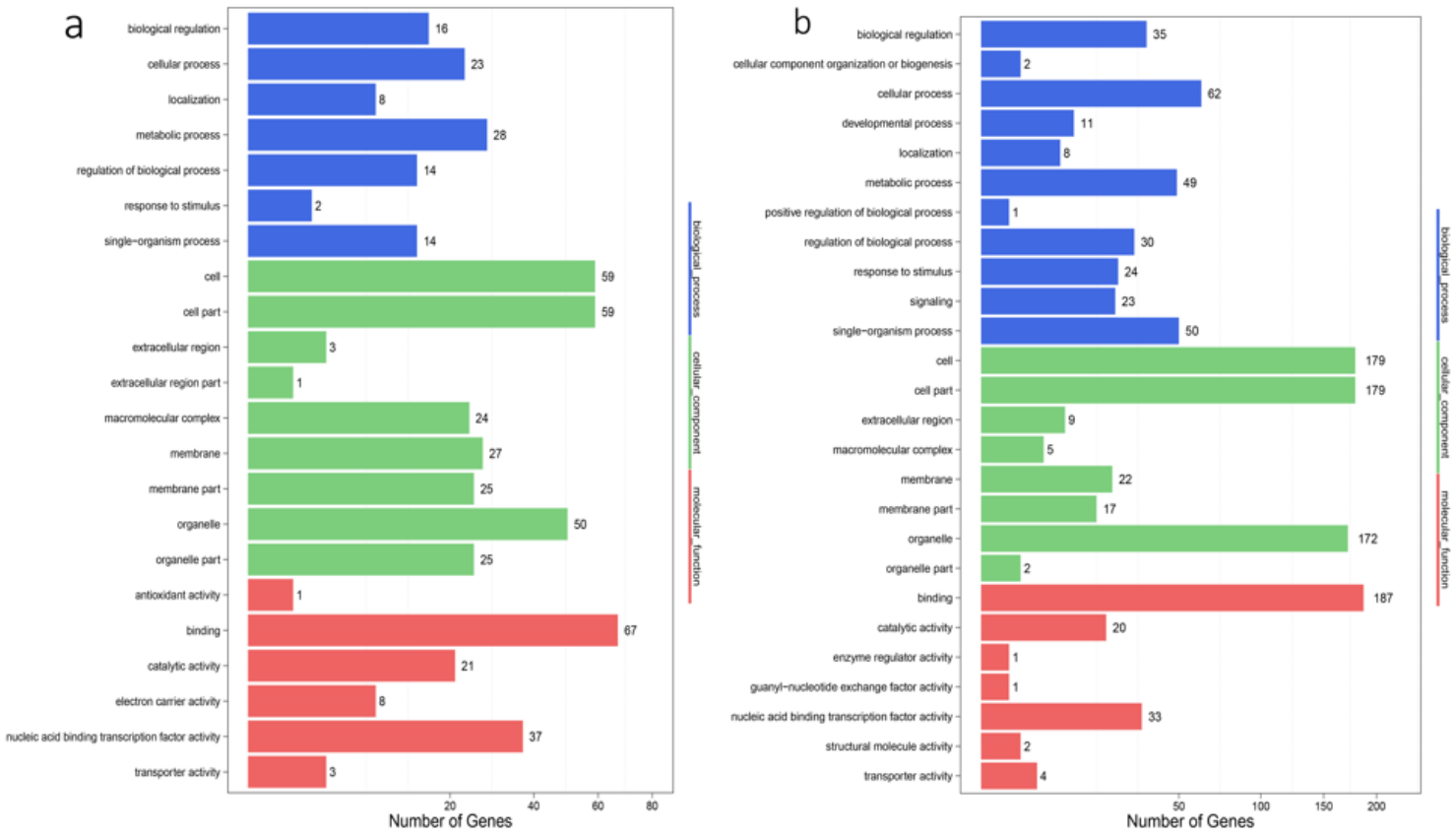

Figure 7

Gene Ontology annotation of DEMs (a) Brazilian banana (b Guijiao No.1

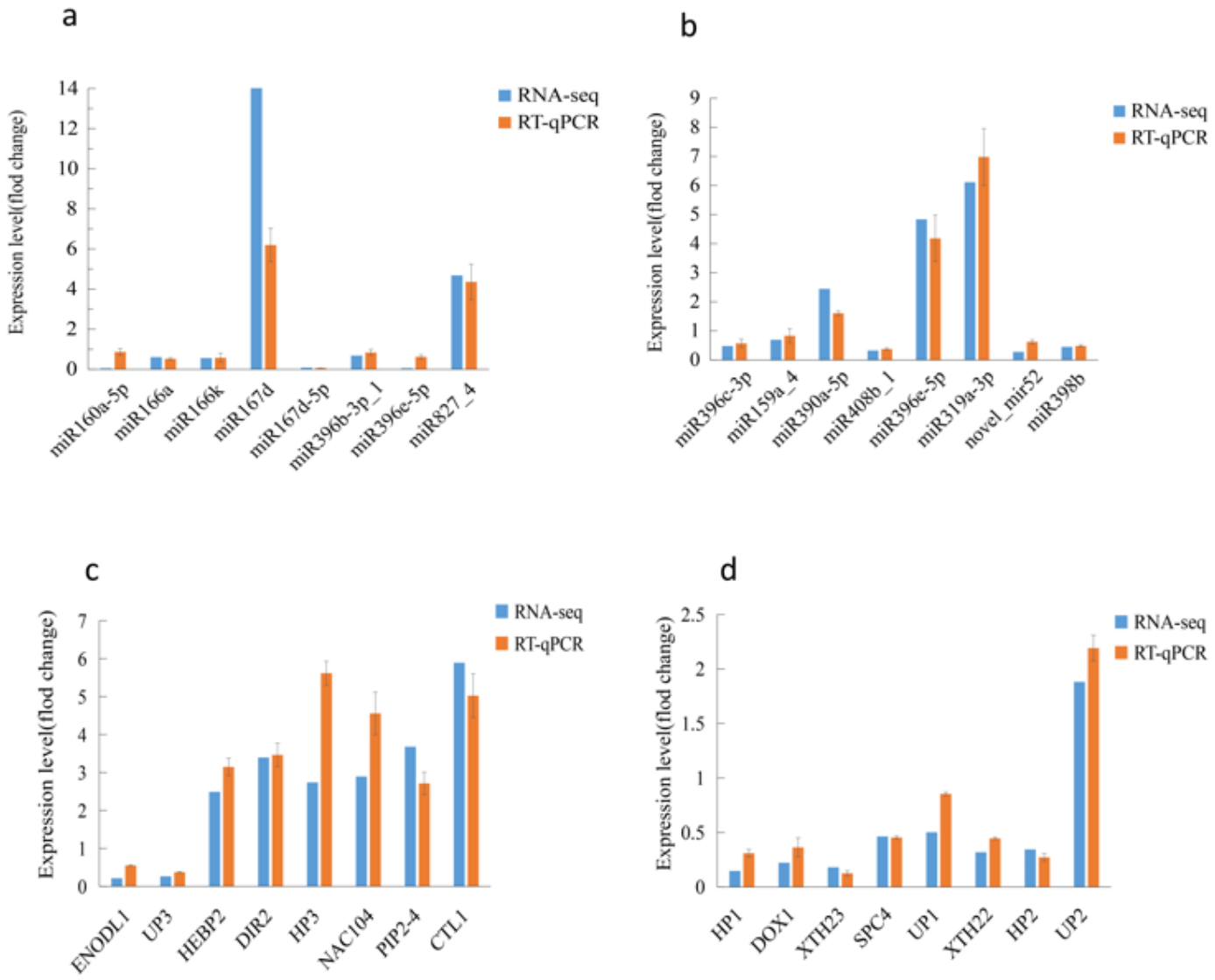

Figure 8

validation of DEMs and DEGs byRT- qPCR (a) Guijiao No.1 DEMs (b)Brazilian banana DEMs (c)Guijiao No.1 DEGs (d)Brazilian banana DEGs

Note:HP,hypothetical protein;UP,uncharacterized protein

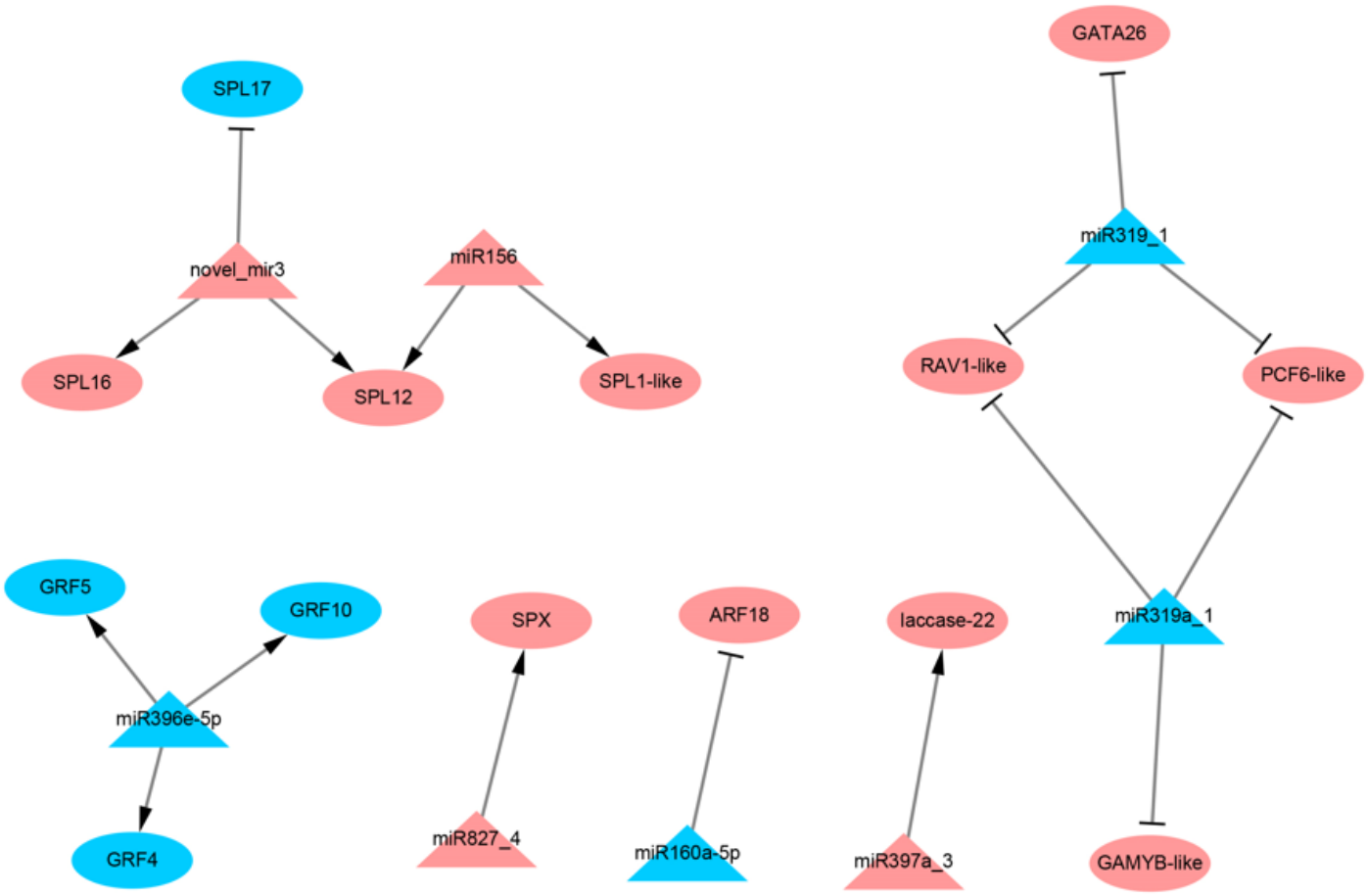

Figure 9

Regulatory network from the integrated analysis of miRNA-mRNA data(Guijiao No.1).Red represents up-regulation and blue represents down-regulation in network, the ellipse represents mRNA, the rectangle represents miRNA.

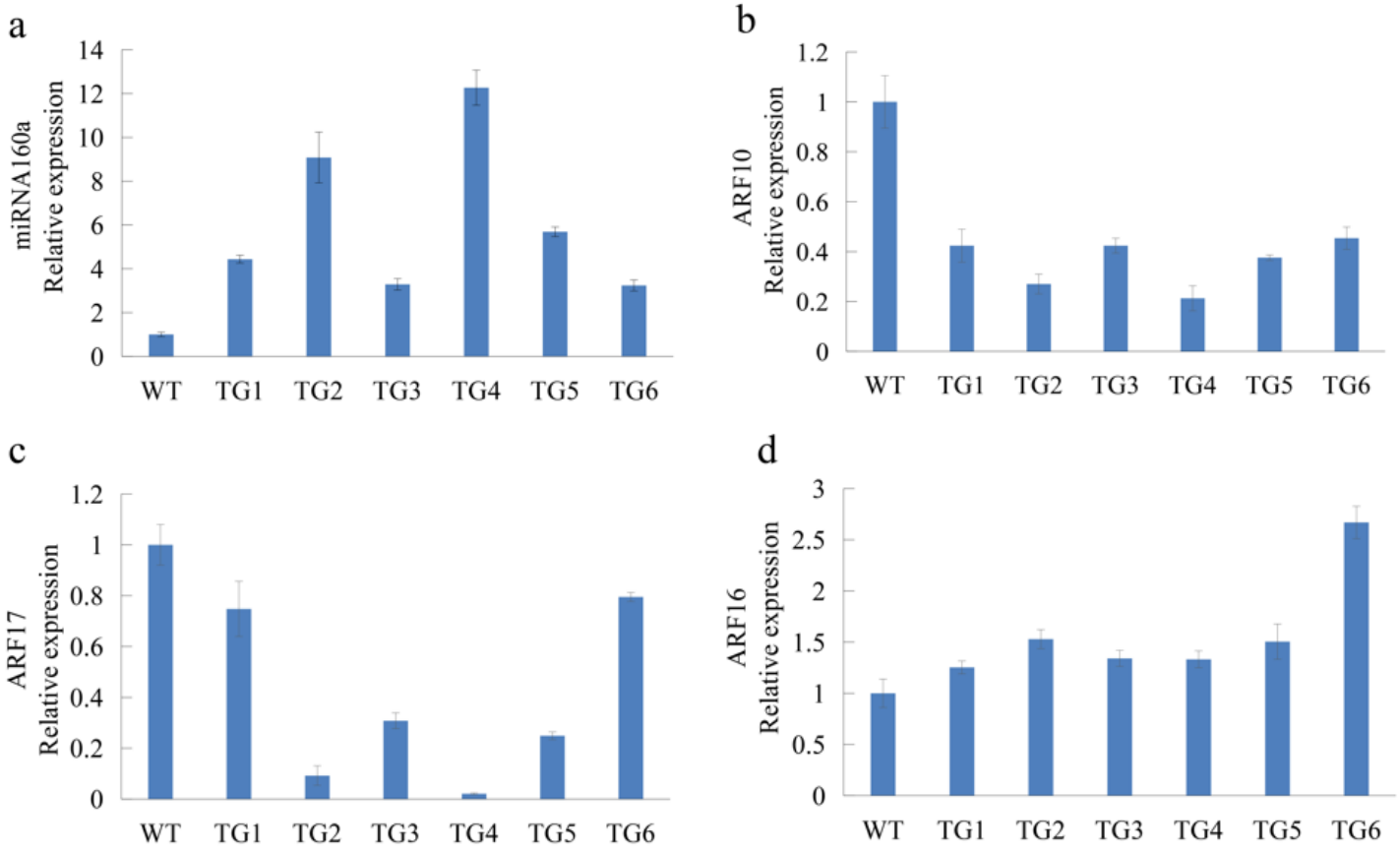

Figure 10

Quantitative expression analysis of transgenic *Arabidopsis* miR160a and target gene.(a) RT-qPCR of miR160a,(b)RT-qPCR of ARF10,(c)RT-qPCR of ARF17,(d)RT-qPCR of ARF16.

WT: wild type TG: miR160a overexpression strain

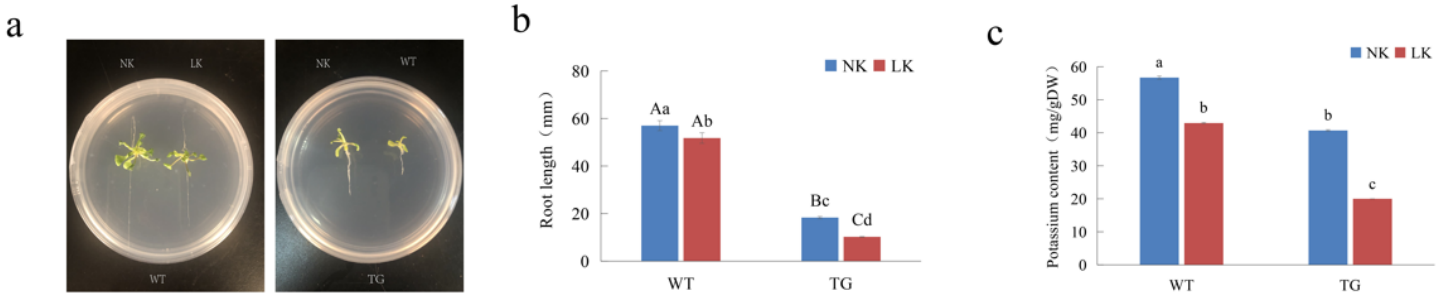

Figure 11

Preliminary functional validation of mi160a by transgenic *Arabidopsis*.(a)Effect of low K stress on the phenotype of *Arabidopsis*.(b)Effect of low K stress on root length of *Arabidopsis*.(c)Effect of low K stress on potassium content in *Arabidopsis*.

Supplementary Files

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- Supplementarymaterial.docx