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 higher 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 assist 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. Analysis of transcription factors showed that they were mainly in the types or classes of MYB, AP-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 between normal and low K treatments. 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 overexpressing miR160a showed reduced tolerance to low K, and inhibited phenotypic traits such as shorter root length, and reduced K accumulation. The overexpressed miR160a had a targeting relationship with ARF10 and ARF16 in Arabidopsis. These results indicate that miR160a may regulate K absorption in bananas through the auxin pathway. This study provides a theoretical basis for further study on the molecular mechanism of banana response to low potassium stress.

Keywords Musa · miRNAs · mRNAs · Potassium deficiency · miR160a

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

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miRNAs (Thornburg et al. 1219). MiR160 acts on ARF10, sion of ARF transcription factor and their corresponding shown that K deficiency can cause changes in the expres-
flowering time by altering FT expression via NaKR1 under sses (Liu et al. 2021). The miR156-SPL3 module modulates negative regulatory role in response to salt and drought stre
Chrysanthemum way under low-K stress (Ye et al. 2021). In
TCA cycle, glycolysis pathway and pentose phosphate path-
mir169h, and miR395a regulatory modules can mediate the TCP4 and miR396 / GRF mediated pathways. The miR164c, be explained by miR444 / MADS box model, miR319 / movement, protein synthesis and osmotic regulation, and resistance to many biotic and abiotic stresses (The mineral nutrition of higher plants 1980; Jin et al. 2011; Jones RALaRGW 1984). K deficiency significantly inhibits the growth and development of bananas (Xu et al. 2019b).
MicroRNAs (miRNAs) are endogenous small non-cod-
ing RNAs, usually 20–24 nucleotides (nts) in length, which negatively regulate gene expression by promoting the degra-
dation of target mRNA or inhibiting its translation (Zhu et al. 2021). 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 of biotic and abiotic stresses (Li et al. 2020; Ning 2019; Song et al. 2016; Zhang et al. 2020a).
miRNAs play a key role in regulating nutrient absorp-
tion 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 (Zhang et al. 2020b). Under low-K stress, miR160a, miR396c and miR169h regulate plant photosynthesis (Liu et al. 2018). 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 (Zhu et al. 2021). In barley, atamiR1432-5p can participate in the Ca²⁺ signaling pathway in response to low-K stress (Zeng et al. 2019). The difference of barley genotypes’ tolerance to low potassium can be explained by miR444 / MADS box model, miR319 / TCP4 and miR396 / GRF mediated pathways. The miR164c, miR169h, and miR395a regulatory modules can mediate the TCA cycle, glycolysis pathway and pentose phosphate pathway under low-K stress (Ye et al. 2021). In Chrysanthemum indicum, the regulatory module of miR396a-GRFs plays a negative regulatory role in response to salt and drought stre-
Measurement of K⁺ content

Fourteen days after treatment, seedlings were harvested for assessments. The plants were divided into shoot and root. Six replicates of each setting were stored in liquid nitrogen for ATP enzyme analysis and RNA extraction (see below), and three replicates of each setting were cured at 105 °C for 15 min and dried at 80 °C for 48 h to constant weight. After weighing for dry weights, the dried tissues were ground into powder, added concentrated HNO₃ and digested for 4 h using Multiwave PRO (Anton Paar) the determination of K concentrations using ICP-OES (Optima 7000DV).

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 Cu²⁺-Mg²⁺-ATPase activity was measured using the Ca²⁺-Mg²⁺-ATPase activity detection Kit (Solarbio, Bei-
ing, China).
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.

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 Oligo dT. DNA probe was used to hybridize rRNA, RNaseH selectively digested the DNA/RNA hybrid chain, and then DNA probe was digested with DNase I, 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 were 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.

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

The filter conditions of DEGs were Q-value (Adjusted P-value) ≤ 0.05, log₂(foldchange)>0. The filtering conditions for DEMs were P-value ≤ 0.05, log₂(foldchange)>0. All DEGs and target genes of DEMs are subjected to Gene Ontology and KEGG Pathway analysis. The P-value was 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.

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

To identify the accuracy and reliability of mRNAs and miRNAs 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: 30 s at 95°C, followed by 40 cycles of 5 s at 95°C and 30 s at 60°C. Each measurement was repeated using three biological replicates with three technical replicates for each of biological replicate. Reference gene: 5S rRNA for miRNA quantification, CAC for miRNA targets quantification, and ACTIN was used for the mRNA quantification. All the primers were listed in Supplementary Table S3.

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 miRNAs were combine with its targeted mRNAs to construct a miRNAs/mRNAs regulatory network. The drawing was passed Cytoscape_v3.8.2.

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(Lin et al. 2021). 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 RT-qPCR. T3 homozygous transgenic lines were used in the experiment.

**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, KNO₃ was changed to 0.05 mmolL⁻¹,KH₂PO₄ was replaced by NH₄H₂PO₄. Samples were collected after 14 days of low-K treatment for the determination of indicators. Potassium content was determined by ICP-OES, and root length was determined by vernier caliper (Li et al. 2017).

**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.

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**Results**

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

Under low-K stress, ‘Guijiao No.1’ (low-K tolerant) showed better than ‘Brazilian banana’ (low-K sensitive). The root system of ‘Guijiao No.1’ was less affected by low-K stress, 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 Ca²⁺/Mg²⁺-ATPase of ‘Guijiao No.1’ was increased significantly (P < 0.05), the root Ca²⁺/Mg²⁺-ATPase of ‘Guijiao No.1’ also was increased significantly (P < 0.05) (Fig. S1).

![Fig. 1](image-url)

Fig. 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 significant difference in the P values of 0.05
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 30,652, 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. S4a). 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. S4c).

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 RNAs were the most abundant type (Fig. S4b). Correlation analysis showed good correlation between samples (Fig. S5).

Analysis of differentially expressed genes (DEGs)

In order to identify DEGs under low-K stress, DEseq2 software was used to set Q-value (Adjusted P-value, 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. 3a). Significant differences in the number of DEGs between the two breeds were due to differences in genotypes. The Venn diagram of DEGs showed that only 5 DEGs were co-expressed, namely 103,982,946, 103,986,668, 103,991,590, BGI_novel_G000350, BGI_novel_G000351 (Fig. 3b). The clustering heat map of the DEGs showed that there were more up-regulated genes in ‘Guijiao No.1’, while more down-regulated genes in ‘Brazilian banana’ (Fig. S2).

In order to better understand the function of DEGs, GO analysis was performed on them (Fig. 4a, b). 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) (Fig. 4b, c).

Analysis of differentially expressed miRNAs (DEMs)

In order to identify miRNAs related to low-K stress, log2(fold change) > 0.5 and \( P < 0.05 \) 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. 5a). The expression heat map of DEMs showed that ‘Guijiao No.1’ had more down-regulated miRNAs than ‘Brazilian banana’ (Fig. 5b).

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

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**Fig. 4** Gene Ontology (GO) and KEGG pathway enrichment of DEGs. a GO of DEGs in Brazilian banana, b GO of DEGs in Guijiao No.1, c Enriched KEGG pathways by DEGs in Brazilian banana, d Enriched KEGG pathways by DEGs in Guijiao No.1
Fig. 5  Number a and heat map b of differential expressed miRNAs

Fig. 6  Gene Ontology annotation of DEMs a Brazilian banana b Guijiao No.1
part, and organelle (Fig. 6). 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).

**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. RT-qPCR of miRNA160a and ARF18 confirmed their expression trends were correct (Fig. 7).

**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 \( \log_{2}(\text{fold change}) \geq 1 \) 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. 8). Among the 8 negative miRNA-mRNA pairs, the expression of miR160a 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. 9a). 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. 9b, c). The expression of ARF16 did not have a negative correlation with miRNA160a (Fig. 9d). 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. 10a, b). 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. 10c).

**Discussion**

Soil potassium deficiency had become an important factor affecting crop yield and quality. Therefore, how to make bananas more effectively utilize potassium resources in the soil was an urgent problem to be solved. 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 bananas was first manifested at the physiological level. The ‘Guijiao No.1’ (low-K tolerant) had better plant height, biomass, K concentration and accumulation under low-K stress. The \( \text{H}^+ / \text{K}^+ -\text{ATPase} \) and \( \text{Ca}^{2+}/\text{Mg}^{2+} -\text{ATPase} \) of ‘Guijiao No.1’ in root that showed an extremely significant increase under low-K stress. Research had confirmed that the tolerance of banana varieties under low-K conditions may be related to ATPase activity (Ye et al. 2017).

The difference in genotype response was secondly manifested at the mRNA level. ‘Guijiao No.1’ (low-K tolerant) had more DEGs responses under low-K+ conditions. The first three functions of ‘Guijiao No.1’ GO analysis mainly focused on cellular anatomical entity, catalytic activity, binding, etc. This was consistent with studies in tomatoes and Chinese yam (Zhao et al. 2018; Zhou et al. 2020). 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 participated in biotic and abiotic stress responses (Dudareva et al. 2013). In recent years, studies in Arabidopsis had 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 (Gish and Clark 2011). The MAPK was located downstream of RLKs and played a central role in biotic and abiotic stress signaling (Nakagami et al. 2005). Protein processing in the endoplasmic reticulum could confer the function of protein signal transduction (Chen et al. 2020). 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 (Zeng et al. 2021).

MiRNAs play a post-transcriptional regulatory role by targeting mRNAs, and their roles in regulating plant nutrient stress have been extensively studied. In this study, ‘Guijiao No.1’ showed more response 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

Fig. 7 validation of DEMs and DEGs by RT-qPCR a Guijiao No.1 DEMs b Brazilian banana DEMs c Guijiao No.1 DEGs d Brazilian banana DEGs e miRNA160a and ARF18 RT-qPCR Note: HP, hypothetical protein; UP, uncharacterized protein
affect cell division, differentiation, elongation, germination and rooting, plant height, branching, flowering and fruiting, etc., and played an important regulatory role in plant growth and development (Wang et al. 2020). 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 isochromoline alkaloids. Among them, tyrosine metabolites, tocopherols, plastoquinone and ubiquitinone were necessary for plant survival (Xu et al. 2019a).

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 mRNA formed 14 pairs of miRNA-mRNAs in the yam tuber regulatory network (Zhou et al. 2020). The Squamosa Promoter-Binding Protein-Like (SPL) gene encoded a plant specific transcription factor, which played a role in flower and fruit development, gibberellin signaling, and sporogenesis (Chen et al. 2010). The feedback interaction of miR156-SPLs ran through the entire plant development process (Negishii et al. 2018). In Arabidopsis, many SPL genes were regulated by miR156 after transcription, and AtSPL9 in turn positively regulated the expression of miR172 (Chen et al. 2010). In this study, 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 (Cui et al. 2018). In this study, miR319_1 acted on the three target genes of GATA26, RAV1, and PCF6. GATA transcription factors were a kind of transcription factors widely existing in eukaryotes. They played an important role in the biological processes of plant light response regulation, chlorophyll synthesis, 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 study (Bhardwaj et al. 2015; Bonthala et al. 2016; Zhang et al. 2015). RAV-like proteins were a subclass of the AP2 protein family and played a key regulatory role in plant stress responses and hormone responses (Yasuaki Kagaya KOaTH 1999). 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) (Martin-Trillo and Cubas 2010; Navaud et al. 2007). 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 (Huo et al. 2019). 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 (Gocal et al. 2001). 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 (Wild type), the TG (Transgenic) 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 (Liu et al. 2010). 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 (Wang et al. 2005). miR160 degraded the target genes ARF16 and ARF10 by cutting...
them, regulating root tip growth and geotropism (Liu et al. 2007). miR160a/b was a key regulator in potatoes, which affected the root structure of plants by cutting the mRNA of StARF10 and StARF16 (Yang et al. 2021). That miR160 was involved in the formation of adventitious roots of apple rootstocks induced by auxin (Meng et al. 2020). miR160 directed soybean nodule development in soybeans (Nizampatnam et al. 2015). miR160-ARF18-mediated peanut (Arachis hypogaea L.) response to salt stress (Tang et al. 2021). 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 (Bustos-Sanmamed et al. 2013). In peanuts, the down-regulation of miR160a may promote the growth of primary roots and lateral roots under potassium-deficient conditions (Li et al. 2021). 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 new insights into the molecular mechanism of banana tolerance to low-K stress.

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Authors’ contributions WC, TD, JL and MW conceived the research; WC, CW and YT designed and conducted the experiments; WC, PL and KC analyzed the data; WC wrote the manuscript; HY, YC and MW revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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

Declarations

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

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