Transcriptome Analysis of Banana (Musa acuminata L.) in Response to Low-Potassium Stress

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Abstract: Potassium (K⁺) is an abundant and important macronutrient for plants. It plays crucial roles in many growth and developmental processes, and growth is inhibited under low −K⁺ conditions. The molecular mechanisms operating under K⁺ starvation have been little reported in banana, which is a non-model plant. We conducted a transcriptome analysis of banana (Musa acuminata L. AAA group, cv. Cavendish) in response to low −K⁺ stress. The phenotypic traits and transcriptomic profiles of banana leaves and roots were compared between low −K⁺ (LK) and normal −K⁺ (NK) groups. The phenotypic parameters for the LK group, including fresh and dry weight, were lower than those for the NK group, which suggested that low −K⁺ stress may inhibit some important metabolic and biosynthetic processes. K⁺ content and biomass were both decreased in the LK group compared to the NK group. Following ribonucleic acid sequencing (RNA-Seq), a total of 26,796 expressed genes were detected in normal −K⁺ leaves (NKL), 27,014 were detected in low −K⁺ leaves (LKL), 29,158 were detected in normal −K⁺ roots (NKR), and 28,748 were detected in low −K⁺ roots (LKR). There were 797 up-regulated differentially expressed genes (DEGs) and 386 down-regulated DEGs in NKL versus LKL, while there were 1917 up-regulated DEGs and 2830 down-regulated DEGs in NKR versus LKR. This suggested that the roots were more sensitive to low −K⁺ stress than the leaves. DEGs related to K⁺ transport and uptake were analyzed in detail. Gene functional classification showed that the expression of genes regarding ABC transporters, protein kinases, transcription factors, and ion transporters were also detected, and may play important roles during K⁺ deficiency.

Keywords: banana; differentially expressed genes; K⁺ transporter; low-potassium stress; transcriptome

1. Introduction

Potassium (K⁺) is one of the most abundant and important macronutrients in high-biomass plants, especially in banana plants [1]. It accounts for 2–10% of the total dry weight in plants, and plays key roles in plant growth and development processes, such as osmoregulation, photosynthetic efficiency via balancing gas exchange, pH adjustment, the cotransport of sugars, activating enzymes, and protein biosynthesis [2–4]. It has been reported that the concentration of K⁺ in soil is usually below 1 mM [5]. However, K⁺ accumulates in plant cells and can reach concentrations of 100 mM [6]. Therefore, many plants experience low −K⁺ stress during their lives [7]. The low −K⁺ stress signals can be transduced across the plasma membrane and into the cytosol, and the K⁺ homeostasis is modulated by K⁺ channels and transporters, which facilitates plant adaptation to K⁺-deficient conditions [8]. When faced with
low-K⁺ conditions, the growth rate slows and can even stop, the leaves of most plants become yellow and old leaves display tip-burn, pathogen susceptibility increases, and fruit yield and quality decreases [9].

Efficient K⁺ uptake and survival under K⁺-starved conditions are of great importance to plants. Ion uptake and transport in plants occur via a complicated signaling network, with a variety of anions and cations participating in salinity stress tolerance. It has been reported that plants maintain a constant level of K⁺ via K⁺ transporters and channels, enabling them to adapt to low –K⁺ conditions [10]. A variety of K⁺ transporters and channels have been described in relation to K⁺ uptake, such as the high-affinity K⁺ transporter/K⁺ uptake transporter (HAK/KUP/KT) family, which includes AtHAK1/5 [11], PpHAK2 [12], AtKUP1 [13], and OsHKT2 [14,15], and K⁺ transporters such as AtAKT1/5 [16] and OsAKT1 [17,18]. These transporters and channels are associated with K⁺ uptake and maintain K⁺ homeostasis in plant cells. Low –K⁺ conditions are a typical form of abiotic stress, and can induce a series of biological responses. Reactive oxygen species (ROS) and plant phytohormones are induced during K⁺ starvation [19,20]. There have been many studies of the molecular mechanisms that occur under K⁺ starvation using model plants such as Arabidopsis and rice. However, these mechanisms have little reported in banana as a classical tropical plant.

RNA sequencing (RNA-Seq) is generally used to analyze the gene expression of particular biological subjects under specific conditions [21]. RNA-Seq can be applied to gene expression analysis, differentially expressed gene (DEG) screening, expression pattern analysis of DEGs, and gene ontology (GO) classification. The Banana Genome Hub is a next-generation information system for Musa genomics (http://banana-genome-hub.southgreen.fr). It is an excellent tool for the study of banana biological processes, given that the sequencing of the banana (Musa acuminate L.) genome was completed in 2012 and characterized further in 2016 [22,23].

In the present study, we used RNA-Seq technology to monitor the transcriptomic profiles of banana leaves and roots in response to a low –K⁺ treatment. We analyzed the functional categorization of DEGs and compared the low –K⁺ gene expression profiles between leaves and roots.

2. Materials and Methods

2.1. Plant Materials and Low –K⁺ Stress Treatment

*Musa* AAA Cavendish (Brazilian banana) tissue-cultured seedlings with four leaves and 10 cm in height were purchased from the DanZhou plantation base company of Hainan Province, China and chosen as the study materials. The seedlings were divided into normal and low –K⁺ groups with 20 plants in each group. The normal group and treated group were placed in a greenhouse in Hainan Province, China with a temperature of 28–35 °C in summer. The plants were watered by Hoagland’s complete nutrient solution and low-potassium (K⁺) culture solution every two days, respectively (Supplemental Material 1) for 60 days until symptoms appeared in treated groups. Each group of plants was divided into an aboveground part (leaves and pseudostems) and an underground part (roots), resulting in four different types of sample: normal –K⁺ leaves (NKL), normal –K⁺ roots (NKR), low –K⁺ leaves (LKL), and low –K⁺ roots (LKR). After collecting, the samples were immediately frozen in liquid nitrogen and stored at –80 °C prior to RNA extraction. The protocols for the use of Hoagland complete culture solution and the low –K⁺ culture solution are provided in Supplemental Material 1.

2.2. Determination of Phenotypic Traits and Measurement of K⁺ Content

The experimental banana seedlings were kept under observation and sampled when they displayed clearly evident symptoms. The total number of leaves, the number of symptomatic leaves, the plant height, the aboveground fresh weight, and the underground fresh weight were recorded and measured. The aboveground and underground tissues were then dried at 80 °C for 48 h, and the dry weight was measured. The dried powder was passed through a 0.25-mm aperture sieve. To analyze
the K⁺ content, dry samples were placed into chemical decomposition tubes with a small amount of water, and 8 mL of concentrated sulfuric acid was then added for overnight. The following day, the samples were incinerated in a chemical decomposition furnace at 250 °C for 30 min; then, the temperature was raised to 400 °C until a brownish-black color appeared in the solution, after about 3 h. The solution was cooled, and 10 drops of 30% H₂O₂ were added and mixed gently before boiling for 5 min. This step was repeated three to five times with the H₂O₂ gradually being removed until the solution became colorless. The solution was then poured into a 100-mL volumetric flask and diluted to 100 mL with double-distilled water. The K⁺ concentration was measured with a flame photometer, with three biological and technological replicates used for K⁺ measurement, and SPSS software (R24.0, IBM, Chicago, IL, USA) and a significant t-test (* \( p < 0.05 \), ** \( p < 0.01 \)) were used to determine the statistical significance.

2.3. RNA-Seq and Bioinformatics Analysis

The RNA-Seq and bioinformatics analysis were entrusted to Shenzhen BGI Tech Company (Shenzhen, China). Six plants of each group were used for RNA extraction with an improved cetyltrimethylammonium bromide (CTAB) method [24]. The quantity and quality of the RNA were done with a spectrophotometer Ultrospec 2100 pro (Amersham Biosciences, Cambridge, UK) and 1% agarose gel electrophoresis. The extracted RNA was first treated with DNase I to degrade any possible DNA contamination. The messenger ribonucleic acid (mRNA) was enriched using oligo (dT) magnetic beads (Invitrogen) according to the manufacturer’s protocol. After mixing with fragmentation buffer, the mRNA was fragmented into short fragments. The first-strand complementary DNA (cDNA) was synthesized using a random hexamer primer and the second-strand cDNA was synthesized by adding buffer, dNTPs, RNase H, and DNA polymerase I. The double-stranded cDNA was purified using magnetic beads. Then, the sequencing adaptors were ligated to the fragments. Finally, the fragments were enriched by polymerase chain reaction (PCR) amplification. The PCR products were measured by quality control (QC) step, and a 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA) and a StepOnePlus Real-Time PCR System (Applied Biosystems Inc., Foster City, CA, USA) were used to qualify and quantify sequences for the sample library. The library products were prepared for sequencing using a HiSeq 2000 system (Illumina, San Diego, CA, USA).

The original image data was transferred into sequence data via base calling, which is defined as raw data or raw reads and saved as a fastq file [25]. Those FASTQ files include the detailed read sequences and the read quality information. In addition, the filtering of raw data is needed to decrease the data noise. After filtering, the remaining reads are called “clean reads” and used for downstream bioinformatics analysis. Clean data were obtained and stored as FASTQ format after filtering by detecting the content of each base to measure the stability of the library and sequencing eligibility. Under the normal circumstances, the nucleotide distribution at each position identifies the content of stable, non-AT or GC separation. Base quality reflects the accuracy, while the sequencing, reagents, sample quality, etc., affect the quality of the base. If the percentage of the bases with low quality (<20) is low, then the sequencing quality of this lane is good. After data quality statistics, clean reads were mapped to reference sequences using Burrows-Wheeler Alignment (BWA) tool [26] and to gene reference using Bowtie [27]. In general, a higher ratio of alignment indicated a closer genetic relationship between the samples and the reference species. The genotype of the reference genome was Musa acuminata (DH-Pahang), and the latest version was improved by a combination of methods and datasets, leading to the release of version 2 of the assembly and gene annotation (https://banana-genome-hub.southgreen.fr) [23]. The process of RNA-Seq and bioinformatics analysis are presented in Figure 1. The raw data of the high-throughput sequence (RNA-Seq) results and processed files have been submitted to the Gene Expression Omnibus (GEO) database of the national center for biotechnology information (NCBI) web site; its accession number is GSE102968.
2.4. Differentially Expressed Genes (DEGs) Screening

In this article, we used RNA-Seq by the FPKM (Fragments Per Kilobase of transcript per Million mapped reads) method to calculate the expression level of the detected genes with the RNASeq by Expectation Maximization (RSEM) software package [28]. The FPKM method is able to eliminate the influence of different gene lengths and sequencing discrepancy on the calculation of gene expression. The formula of the FPKM method is shown as follows: $\text{FPKM} = \frac{10^6 \, C}{NL/10^3}$, which is given to be the expression of gene A; here, $C$ is the number of fragments that are uniquely aligned to gene A, $N$ is the total number of fragments that are uniquely aligned to all genes, and $L$ is the number of bases on gene A. In order to identify the low potassium-responsive genes, the DEGs were analyzed between the normal and treatment groups using $p$-value $<0.05$ and $|\log_{2} \text{Ratio}| \geq 1$ as thresholds. The significance of the DEGs was determined by using the false discovery rate (FDR) control method to justify the $p$-value [29].

2.5. Quantitative Real-Time PCR (qRT-PCR) Analysis

To validate the reliability and repeatability of the RNA-Seq results, 15 genes were selected for identification by qRT-PCR assays (three technological replicates of each gene). Detailed information about these genes is provided in Supplemental Material 2. Tissues of six banana seedlings of each group were mixed and used for RNA extraction with the improved cetyltrimethylammonium bromide (CTAB) method [24]. Total RNA was treated with DNase I and RNase-free (Thermo Fisher Scientific, Waltham, MA, USA) to eliminate genomic DNA contamination. For first-strand cDNA synthesis, 1.0 $\mu$L of cDNA template, 0.8 $\mu$L of PCR forward primer (10 $\mu$M), 0.8 $\mu$L of PCR reverse primer (10 $\mu$M), and 2 $\mu$L of oligo (dT)18 primers (Revert Aid First Strand cDNA Synthesis Kit, #K1621, Thermo Fisher Scientific, Waltham, MA, USA) was used in the PCR reaction system on a 7500 RT-PCR System (Applied Biosystems, Waltham, MA, USA) according to the manufacturer’s protocols. SYBR® Premix Ex Taq II (Takara, Dalian, China) was used in the PCR reaction system containing 10 $\mu$L of SYBR® Premix Ex Taq II (Takara, Tli RNase Plus, Dalian, China) (2×), 2 $\mu$L of cDNA template, 0.8 $\mu$L of PCR forward primer (10 $\mu$M), 0.8 $\mu$L of PCR reverse primer (10 $\mu$M), and 0.2 $\mu$L of 5× Premix Enzyme (Takara, Dalian, China). The significance of the DEGs was determined by using the false discovery rate (FDR) control method to justify the $p$-value [29].
µM), 0.4 µL of ROX reference DyeII (50×), and 6 µL of nuclease-free water. The PCR amplification program was as follows: 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, and 60 °C for 1 min. The $2^{-\Delta\Delta CT}$ method was used for qPCR data analysis, in which $\Delta CT(\text{test}) = CT(\text{target, test}) - CT(\text{ref, test})$, $\Delta CT(\text{calibrator}) = CT(\text{target, calibrator}) - CT(\text{ref, calibrator})$, and the expression ratio of the experimental and control groups was $2^{-\Delta CT}$. The primers of the selected genes were designed using Primer Premier 6 (www.premierbiosoft.com). The amplification of Actin1 (GenBank Accession No. AF246288, submitted by National Taiwan University, Taipei, China) was used as an internal reference gene control for data normalization [30].

3. Results

3.1. K⁺ Deficiency Inhibits the Growth of Banana Seedlings

In the nutriculture experiments with low K⁺ stress, at the early stage (20 days), the edges of the leaves of the banana plants in the low K⁺ group (LK) were slightly yellow, as though they had been burned, compared with those of the normal K⁺ group (NK). However, there were no obvious differences in the phenotypic characteristics of plant height or number of leaves (Table 1). After 60 days, the lower leaves of the seedlings in the LK group became yellow, with brown stains, as though boiling water had been poured over them (the red arrow in Figure 2A). Then, the lower leaves became wilted, and the total number of leaves was less than that in the NK group (Figure 2B). The LK plants were thin and short, with a small leaf area and fewer roots (Figure 2C). These symptoms indicated that stress due to K⁺ deficiency dramatically inhibited the growth and development of banana seedlings.

To investigate the changes between the NK and LK treatments, each plant was divided into an aerial part (leaves and pseudostems) and an underground part (roots). The phenotypic traits of each group were measured, which contained 20 banana seedlings. The phenotypic traits including leaf numbers, plant height, and fresh and dry weights. Spearman analysis was used for correlation analysis of the statistical variables with SPSS software. The results are shown in Table 1.

There were significant differences between the NK and LK treatments in terms of total leaf number and the number of leaves showing symptoms. The fresh and dry weights of the aerial and underground parts in the LK group were significantly less than those in the NK group. This indicated that low K⁺ stress significantly inhibited the growth and development of the banana leaf, pseudostem, and root, and led to a decrease in the biomass of banana plants. We also measured the K⁺ content of the aerial and underground parts of the plants in the NK (NKL and NKR) and LK (LKL and LKR) treatments, respectively. SPSS software was applied to the results (Figure 3), which confirmed that the K⁺ content in the LK group was significantly lower than that in the NK group, indicating that a restricted K⁺ supply affected K⁺ uptake and K⁺ content in banana tissue. In conclusion, the K⁺ content and biomass index of banana seedlings decreased under the stress of low K⁺ conditions.

Table 1. Comparison of phenotypic traits between normal K⁺ (NK) and low K⁺ (LK) treatments.

<table>
<thead>
<tr>
<th></th>
<th>Total Leaf Number</th>
<th>Number of Leaves Showing Symptoms</th>
<th>Plant Height/cm</th>
<th>Fresh weight of Aerial Part/g</th>
<th>Fresh Weight of Underground Part/g</th>
<th>Dry Weight of Aerial Part/g</th>
<th>Dry Weight of Underground Part/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>NK</td>
<td>7.40 ± 0.82</td>
<td>1.55 ± 0.68</td>
<td>15.12 ± 0.82</td>
<td>44.69 ± 4.56</td>
<td>21.38 ± 2.96</td>
<td>6.02 ± 1.31</td>
<td>3.35 ± 0.47</td>
</tr>
<tr>
<td>LK</td>
<td>6.20 ± 0.89</td>
<td>2.00 ± 0.72</td>
<td>11.77 ± 1.13</td>
<td>26.38 ± 4.54</td>
<td>11.67 ± 2.57</td>
<td>3.08 ± 0.76</td>
<td>1.67 ± 0.49</td>
</tr>
</tbody>
</table>

Significance level: ab indicates a significant difference; aa/bb indicates no significant difference.
Figure 2. Phenotypes of banana seedlings under normal and low-potassium (K\(^+\)) conditions. The photographs show a comparison of leaves (A, B) and roots (C) between normal –K\(^+\) (NK) and low–K\(^+\) (LK) treatments. Bar = 5 cm.

Figure 3. K\(^+\) content of plants under different treatments: normal –K\(^+\) leaves (NKL) and low –K\(^+\) leaves (LKL) and normal –K\(^+\) roots (NKR) and low –K\(^+\) roots (LKR). Error bars with different letters indicate significant differences (three technical replicates, \(p < 0.05\)).
3.2. RNA Sequencing, Alignment, and Bioinformatics Analysis in Banana Leaves and Roots

To investigate the differences in gene expression between the normal –K⁺ (NK) and low –K⁺ (LK) treatments in the leaves and roots of banana plants, NKL, LKL, NKR, and LKR samples were selected for RNA-Seq. From the four cDNA libraries, a total of 48,767,309 raw reads were generated (Table 2). After the removal of low quality sequences and short reads, 24,209,199 (49.6% of raw data) clean reads were selected for aligned to the banana reference genome. There were 26,796 expressed genes and 33,388 expressed transcripts in NKL, 27,014 expressed genes and expressed transcripts 33,469 in LKL, 29,158 expressed genes and 35,896 expressed transcripts in NKR, and 28,748 expressed genes and 35,380 expressed transcripts in LKR (Table 2).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Raw Reads</th>
<th>Clean Reads</th>
<th>Genome Map Rate</th>
<th>Expressed Genes</th>
<th>Expressed Transcripts</th>
</tr>
</thead>
<tbody>
<tr>
<td>NKL</td>
<td>12,191,789</td>
<td>12,121,980</td>
<td>84.01%</td>
<td>26,796</td>
<td>33,388</td>
</tr>
<tr>
<td>LKL</td>
<td>12,191,898</td>
<td>12,108,969</td>
<td>84.81%</td>
<td>27,014</td>
<td>33,469</td>
</tr>
<tr>
<td>NKR</td>
<td>12,191,778</td>
<td>12,121,219</td>
<td>82.63%</td>
<td>29,158</td>
<td>35,896</td>
</tr>
<tr>
<td>LKR</td>
<td>12,191,844</td>
<td>12,087,980</td>
<td>81.73%</td>
<td>28,748</td>
<td>35,380</td>
</tr>
<tr>
<td>Total</td>
<td>48,767,309</td>
<td>24,209,199</td>
<td>-</td>
<td>111,716</td>
<td>138,133</td>
</tr>
</tbody>
</table>

We analyzed each NK–LK pair with the R package and constructed scatter plots of all the expressed genes, in which different colors were used to represent up-regulated, down-regulated, or non-regulated genes. The DEGs were analyzed between the normal and treatment groups using p-value < 0.05 and |log2 Ratio| ≥ 1 as thresholds. The significance of the DEGs was determined by using the false discovery rate (FDR) ≤ 0.001 to justify the p-value [29]. Figure 4 shows the results, with the x-axis and y-axis representing the log2 value of gene expression. The screening conditions are given in the figure legend (Figure 4).

![Gene Expression Level (NKL-Vs-LKL)](image1)

**Figure 4.** Scatter diagram showing all of the expressed genes. Blue indicates down-regulated genes under the LK treatment, orange indicates up-regulated genes under the LK treatment, and brown indicates non-regulated genes under the LK treatment. Screening conditions: false discovery rate (FDR) ≤ 0.001; abs (log2(Y/X)) ≥ 1).

The genes that were only expressed in NKL, LKL, NKR, or LKR were defined as specifically expressed genes (SEGs). The number of specifically expressed genes in the LK samples was greater than that in the NK samples (Figure 5A); the low –K⁺ treatment resulted in an increase in the number
of specifically expressed genes. There were 797 up-regulated DEGs and 386 down-regulated DEGs in NKL versus LKL, whereas there were 1917 up-regulated DEGs and 2830 down-regulated DEGs in NKR versus LKR (Figure 5B). There were 266 differentially up-regulated genes and 33 differentially down-regulated genes that were common to both leaves and roots (Figure 6). The statistical analysis suggested that these genes may be expressed conservatively in banana and play important roles under low \( -K^+ \) conditions.

![Bar chart showing the number of species expressed genes and significantly up-regulated and down-regulated genes in different samples.](image)

**Figure 5.** (A) Number of specifically expressed genes in different samples; (B) significantly up-regulated and down-regulated genes in the two NK–LK pairs.
Figure 6. Venn diagram showing the number of overlapping and non-overlapping genes differentially expressed in leaves and roots.

All of the DEGs were mapped to the terms of the gene ontology (GO) database (http://www.geneontology.org), which calculated the gene numbers of each term. Then, the hypergeometric test was used to find the enriched GO terms of DEGs compared to the whole genome. $p$-Value was calculated by Gene Ontology TermFinder, and corrected by Bonferroni. GO terms whose corrected $p$-value $\leq 0.05$ were defined as prominent enriched GO terms. In leaves, the GO analysis identified 34, 122, and 197 terms, which contained 1472, 1760, and 2495 genes relevant to cellular components, molecular functions, and biological processes, respectively. In roots, there were 100, 224, and 404 terms, which contained 8483, 9038, and 12,541 genes relevant to cellular components, molecular functions, and biological processes, respectively. Genes that had a cluster frequency $\geq 20\%$ were selected and compared among all of the GO categories in leaves and roots (Figure 7).

In all of the samples of leaves and roots, the GO terms for cells and cell parts were highly represented in the cellular components, catalytic activity was highly represented in the molecular functions, and metabolic processes were highly represented in the biological processes. Interestingly, some GO terms were represented in roots, but not in leaves. These data indicated that the DEGs for cells, catalytic activity, and metabolic processes were most relevant to low $-K^+$ stress.
Figure 7. Gene ontology (GO) function analysis of leaves ((A): normal $-K^+$ leaves (NKL) vs. low $-K^+$ leaves (LKL)) and roots ((B): normal $-K^+$ roots (NKR) vs. low $-K^+$ roots (LKR)) of banana seedlings in a low $-K^+$ stress experiment in terms of cellular components, molecular functions, and biological processes.
3.3. DEGs Related to K⁺ Transport and Uptake in Roots and Leaves

In plants, the HAK/KUP/KT system, the active K⁺ transporter (AKT) family, hyperpolarization-activated cyclic nucleotide-gated K⁺ channel (HCN channels), cation/Ca²⁺ exchangers (CCX; also called NCLX), and calcineurin B-like protein (CBL)-interacting protein kinase (CIPK) genes are all associated with K⁺ transport, uptake, and cellular ionic homeostasis [13,31–34]. From a combined Kyoto Encyclopedia of Genes and Genome (KEGG), GO, and Blast NR databases analysis, 10 genes (three down-regulated, seven up-regulated) related to K⁺ in leaves and 21 genes (eight down-regulated, 13 up-regulated) related to K⁺ in roots were detected (Table 3 and Figure 8). The heatmap was generated by a tool in http://www.omicshare.com/tools/.

Among the DEGs, Ma05_g07130 (K⁺ transporter 1, isoform X2), which is involved in the K⁺ uptake protein (KUP) system, was up-regulated by 3.6-fold of the log2 ratio (LKL/NKL) in leaves and by 2.9-fold of the log2 ratio (LKR/NKR) in roots. This indicates that Ma05_g07130 was up-regulated by low -K⁺ stress in both leaves and roots. It has been reported that AtKUP1 is an Arabidopsis gene encoding a high-affinity K⁺ transporter [13]. Thus, the identified banana gene Ma05_g07130 may be playing important roles in K⁺ transport and uptake as an ion transporter.

Previous studies have proven that AtAKT1 plays critical roles in the inward-rectifying channel and K⁺ uptake, and OsAKT1 represents the dominant salt-sensitive K⁺ uptake channel in rice roots [17,18,35]. OsAKT5 is necessary for rice to take up K⁺ and transport it from roots to shoots at low K⁺-supply levels [36]. PpHAK2 is a possibly K⁺–H⁺ symporter or K⁺/H⁺ antiporter, and performs the second function of HAK transporters [12]. In our results, we detected three KT-like DEGs (Ma05_g24780, Ma05_g07130, and Ma02_g04540) in leaves, six KT-like DEGs (Ma05_g07130, Ma07_g21520, Ma03_g06090, Ma07_g12190, Ma09_g11150, and Ma11_g16690) in roots, two KAT-like DEGs (Ma07_g09180 and Ma10_g27850) in leaves, and three KAT-like DEGs (Ma08_g23650, Ma05_g09470, and Ma06_g28610) in roots. We also found a probable voltage-gated K⁺ channel in roots (Ma09_g24000). These DEGs may play important roles in K⁺ uptake and transport in banana seedlings in response to low −K⁺ treatment.

As an important cytosolic signaling ion, Ca²⁺ affects many cellular processes [37]. The cation/Ca²⁺ exchanger superfamily is widely present in microorganisms, animals, and plants [33]. In plants, CCX superfamily genes play important roles in cellular Ca²⁺ homeostasis, and exchange sodium (Na⁺) or lithium (Li⁺) for Ca²⁺ [34,38]. In this study, two differentially expressed cation/Ca²⁺ exchanger genes were detected in NKL versus LKL and NKR versus LKR. The first was cation/Ca²⁺ exchanger 1-like (Ma08_g20660), which was up-regulated 2.4-fold in LKL, and the other was cation/Ca²⁺ exchanger 5 (Ma05_g18280), which was down-regulated 2.9-fold in LKR. These two genes may play critical roles in maintaining cellular Ca²⁺ levels and the ionic balance of banana plants during low −K⁺ conditions.
### Table 3. Differentially expressed genes (DEGs) related to potassium in leaves and roots.

(A) Differentially Expressed Genes Related to Potassium in Leaves

<table>
<thead>
<tr>
<th>Gene ID</th>
<th>NKL-FPKM</th>
<th>LKL-FPKM</th>
<th>log2 Ratio (LKL/NKL)</th>
<th>Up–Down-Regulation (LKL/NKL)</th>
<th>Gene Function Annotation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ma03_g24780</td>
<td>2.66</td>
<td>0.44</td>
<td>−2.60</td>
<td>Down</td>
<td>Potassium transporter 13 [Musa acuminata subsp. malaccensis]</td>
</tr>
<tr>
<td>Ma07_g09180</td>
<td>9.3</td>
<td>2.77</td>
<td>−1.75</td>
<td>Down</td>
<td>Potassium channel KAT3-like isoform X2 [Musa acuminata subsp. malaccensis]</td>
</tr>
<tr>
<td>Ma11_g04530</td>
<td>77.06</td>
<td>36.94</td>
<td>−1.06</td>
<td>Down</td>
<td>CBL-interacting protein kinase 1 [Musa acuminata subsp. malaccensis]</td>
</tr>
<tr>
<td>Ma05_g07130</td>
<td>1.66</td>
<td>20.65</td>
<td>3.64</td>
<td>Up</td>
<td>Potassium transporter 1 isoform X2 [Musa acuminata subsp. Malaccensis]</td>
</tr>
<tr>
<td>Ma08_g20660</td>
<td>3.97</td>
<td>21.49</td>
<td>2.44</td>
<td>Up</td>
<td>Cation/calcium exchanger 1–like [Musa acuminata subsp. malaccensis]</td>
</tr>
<tr>
<td>Ma02_g04540</td>
<td>4.92</td>
<td>11.5</td>
<td>2.22</td>
<td>Up</td>
<td>Potassium transporter 11 [Musa acuminata subsp. malaccensis]</td>
</tr>
<tr>
<td>Ma10_g27850</td>
<td>0.2</td>
<td>3.22</td>
<td>4.01</td>
<td>Up</td>
<td>Potassium channel KAT3–like [Musa acuminata subsp. malaccensis]</td>
</tr>
</tbody>
</table>

(B) Differentially Expressed Genes Related to Potassium in Root

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>Ma03_g06090</td>
<td>5.79</td>
<td>1</td>
<td>−2.53</td>
<td>Down</td>
<td>Potassium transporter 6-like [Musa acuminata subsp. malaccensis]</td>
</tr>
<tr>
<td>Ma07_g12190</td>
<td>7.8</td>
<td>1.74</td>
<td>−2.16</td>
<td>Down</td>
<td>Potassium transporter 17 isoform X3 [Musa acuminata subsp. malaccensis]</td>
</tr>
<tr>
<td>Ma09_g24000</td>
<td>163.17</td>
<td>71.21</td>
<td>−1.20</td>
<td>Down</td>
<td>Voltage-gated potassium channel subunit beta [Musa acuminata subsp. malaccensis]</td>
</tr>
<tr>
<td>Ma06_g28610</td>
<td>6.23</td>
<td>2.73</td>
<td>−1.19</td>
<td>Down</td>
<td>Potassium channel KAT2-like isoform X5 [Musa acuminata subsp. malaccensis]</td>
</tr>
<tr>
<td>Ma05_g18280</td>
<td>11.8</td>
<td>5.44</td>
<td>−1.12</td>
<td>Down</td>
<td>Cation/calcium exchanger 5 [Musa acuminata subsp. malaccensis]</td>
</tr>
<tr>
<td>Ma09_g11150</td>
<td>12.07</td>
<td>5.59</td>
<td>−1.11</td>
<td>Down</td>
<td>Potassium transporter 7-like [Musa acuminata subsp. malaccensis]</td>
</tr>
<tr>
<td>Ma11_g16690</td>
<td>8.55</td>
<td>4.02</td>
<td>−1.09</td>
<td>Down</td>
<td>Probable potassium transporter 11 [Musa acuminata subsp. malaccensis]</td>
</tr>
<tr>
<td>Ma06_g26140</td>
<td>4.41</td>
<td>0.001</td>
<td>−12.11</td>
<td>Down</td>
<td>CBL-interacting protein kinase 11 [Musa acuminata subsp. malaccensis]</td>
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<tr>
<td>Ma05_g07130</td>
<td>0.37</td>
<td>2.73</td>
<td>2.88</td>
<td>Up</td>
<td>Potassium transporter 1 isoform X2 [Musa acuminata subsp. malaccensis]</td>
</tr>
<tr>
<td>Ma08_g23650</td>
<td>1.65</td>
<td>7.88</td>
<td>2.26</td>
<td>Up</td>
<td>Potassium channel KOR1-like [Musa acuminata subsp. malaccensis]</td>
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<tr>
<td>Ma07_g21520</td>
<td>6.71</td>
<td>29.6</td>
<td>2.14</td>
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<td>Potassium transporter 6-like isoform X2 [Musa acuminata subsp. malaccensis]</td>
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<td>Ma05_g09470</td>
<td>22.34</td>
<td>59.24</td>
<td>1.41</td>
<td>Up</td>
<td>Potassium channel KAT4-like [Musa acuminata subsp. malaccensis]</td>
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<tr>
<td>Ma06_g34240</td>
<td>0.88</td>
<td>4.47</td>
<td>2.34</td>
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<td>CBL-interacting serine/threonine-protein kinase 29 [Musa acuminata subsp. malaccensis]</td>
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<tr>
<td>Ma06_g28510</td>
<td>3.15</td>
<td>14.66</td>
<td>2.22</td>
<td>Up</td>
<td>CBL-interacting serine/threonine-protein kinase 12 [Musa acuminata subsp. malaccensis]</td>
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<tr>
<td>Ma07_g23630</td>
<td>51.84</td>
<td>228.13</td>
<td>2.14</td>
<td>Up</td>
<td>CBL-interacting protein kinase 2 [Musa acuminata subsp. malaccensis]</td>
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<tr>
<td>Ma01_g17400</td>
<td>12</td>
<td>39.95</td>
<td>1.74</td>
<td>Up</td>
<td>CBL-interacting protein kinase 1 [Musa acuminata subsp. malaccensis]</td>
</tr>
<tr>
<td>Ma04_g29510</td>
<td>24.94</td>
<td>74.58</td>
<td>1.58</td>
<td>Up</td>
<td>CBL-interacting protein kinase 23 [Musa acuminata subsp. malaccensis]</td>
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<td>Ma07_g29120</td>
<td>91.26</td>
<td>249.13</td>
<td>1.45</td>
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<td>CBL-interacting protein kinase 6 [Musa acuminata subsp. malaccensis]</td>
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<tr>
<td>Ma03_g14540</td>
<td>10.2</td>
<td>26.84</td>
<td>1.39</td>
<td>Up</td>
<td>CBL-interacting serine/threonine-protein kinase 1 [Musa acuminata subsp. malaccensis]</td>
</tr>
<tr>
<td>Ma04_g15200</td>
<td>28.57</td>
<td>66.81</td>
<td>1.33</td>
<td>Up</td>
<td>CBL-interacting protein kinase 19 [Musa acuminata subsp. malaccensis]</td>
</tr>
<tr>
<td>Ma08_g10700</td>
<td>5.38</td>
<td>12.27</td>
<td>1.19</td>
<td>Up</td>
<td>CBL-interacting protein kinase 1 [Musa acuminata subsp. malaccensis]</td>
</tr>
</tbody>
</table>

FPKM: Fragments Per Kilobase of transcript per Million mapped reads; KAT: K\(^+\) channel from *Arabidopsis thaliana*; CBL: calcineurin B-like protein; KOR: K\(^+\)-outward-(KOR) channels.
Figure 8. Differentially expressed genes (DEGs) related to K⁺ transport and uptake in roots and leaves. Red indicates up-regulated expression, while green indicates down-regulated expression.

The Ca²⁺ ion is a ubiquitous second messenger in plant signal transduction in response to various biotic and abiotic stresses and developmental stimuli [39]. Plants recognize the Ca²⁺ signal through Ca²⁺ sensors, such as calmodulins, calmodulin-like proteins (CMLs), CBLs, and Ca²⁺-dependent protein kinases (CDPKs) [40]. Calcium ion sensors (e.g., CBLs) and their targets (e.g., CIPKs) interact with each other as a CBL–CIPK Ca²⁺ complex, which activates gene expression downstream of the signal transduction pathway in response to stress conditions [5,41]. It has been reported that the CBL–CIPK complex is related to K⁺ uptake by the modulation of AKT channels in rice and Arabidopsis thaliana [16,18]. Two CBL/CIPK pairs were identified in grape vine, which could activate the inward K⁺ channel VvK1.2 [42]. Based on a KEGG pathway analysis and GO function analysis, three up-regulated (Ma07_g17570, Ma07_g05310, and Ma09_g04710) CIPK DEGs and one down-regulated (Ma11_g04530) CIPK DEG were detected in leaves, and nine up-regulated (Ma06_g34240, Ma06_g28510, Ma07_g23630, Ma01_g17400, Ma07_g17570, Ma07_g19510, Ma05_g07130, Ma10_g27850, and Ma08_g28510) CIPK DEGs and one down-regulated (Ma06_g26140) CIPK DEG were detected in roots in this study. We conducted a phylogenetic analysis of these CBL family DEGs in banana leaves and roots by MEGA7.0 (Figure 9). Under low −K⁺ conditions, these DEGs participate in K⁺ uptake and transport in banana to a substantial extent.
Transcription factors are proteins that bind to specific DNA sequences or interact with other transcription factor proteins, thereby controlling the transcription rate of genetic information from DNA to mRNA. Previous studies have shown that MYB-type, bHLH, AP2/ERF, NAC, and WRKY transcription factor proteins, thereby controlling the transcription rate of genetic information from DNA to mRNA. Previous studies have shown that MYB-type, MYB-related, bHLH, AP2-EREBP, WRKY, and NAC transcription factors comprised the top six classes of transcription factor genes differentially expressed in banana leaves and roots (Figure 10). These transcription factors may be among the most critical factors affecting the growth and development of banana seedlings under K⁺-deficient conditions.

**Figure 9.** Phylogenetic analysis of differentially expressed genes (DEGs) of the calcineurin B-like protein (CBL) family in banana leaves and roots.

**3.4. Transcription Factors (TFs) Analysis in Banana Leaves and Roots**

Transporters and channels have important roles in the accumulation of nutrient elements from the soil by plants, and in the maintenance of homeostasis. How the transporters and channels are regulated by upstream transcription factors is of critical importance. Transcription factors are transcriptional regulators in plants and form an integral part of the signaling networks that modulate many plant processes. Transcription factors are proteins that bind to specific DNA sequences or interact with other transcription factor proteins, thereby controlling the transcription rate of genetic information from DNA to mRNA. Previous studies have shown that MYB-type, bHLH, AP2/ERF, NAC, and WRKY transcription factors play important roles in regulating K⁺, Na⁺, and phosphate (PO₄³⁻) transport and salt tolerance in rice and Arabidopsis thaliana [43–49]. Our results revealed that genes encoding MYB, MYB-related, bHLH, AP2-EREBP, WRKY, and NAC transcription factors comprised the top six classes of transcription factor genes differentially expressed in banana leaves and roots (Figure 10). These transcription factors may be among the most critical factors affecting the growth and development of banana seedlings under K⁺-deficient conditions.

**Figure 10.** The top six classes of transcription factor genes differentially expressed in banana leaves and roots.
3.5. Validation of Gene Expression by qRT-PCR

To validate the reliability and repeatability of gene expression in RNA-Seq, a total of 15 genes were selected randomly for identification by qRT-PCR assays (three technical replicates of each gene). The results of the qRT-PCR analysis for these genes mostly matched the expression patterns found by RNA-Seq analysis (Figure 11), which indicated that the RNA-Seq results were reliable.

![Figure 11](image-url)

Figure 11. Validation of gene expression by quantitative real-time polymerase chain reaction (qRT-PCR). (A) Ma02_g01130; (B) Ma08_g01650; (C) Ma06_g32750; (D) Ma01_g16930; (E) Ma04_g36800; (F) Ma09_g07970; (G) Ma04_g25840; (H) Ma03_g32970; (I) Ma06_g17380; (J) Ma10_g00760; (K) Ma03_g30510; (L) Ma01_g00720; (M) Ma08_g01650; (N) Ma10_g22540; (O) Ma09_g07970. The gene expression levels were measured by qRT-PCR and compared with RNA-sequencing (RNA-Seq) results. The histograms represent the fold changes of genes (low K+/normal K+: LK/NK) by qRT-PCR, while the line charts represent gene expression by the log2 Ratio (Fragments Per Kilobase of transcript per Million mapped reads (FPKM) of LK/FPKM of NK) in RNA-Seq. All of the genes selected for qRT-PCR analysis were analyzed in three technological replicates. Bars represent the ± SE of three replicated experiments ($\Delta C_T\text{(test)} = C_T\text{(target, test)} - C_T\text{(ref, test)}$). Details of the genes selected for qRT-PCR are provided in Supplemental Material 2.
4. Discussion

Potassium is the most important and abundant cation in plant cells, playing critical roles in plant physiological function [50]. Reports showed that potassium mainly participates in the electrical neutralization of inorganic and organic anions and macromolecules, pH homeostasis, the control of membrane electrical potential, the regulation of cell osmotic pressure, optimal protein synthesis, and photosynthesis [51]. In addition, as cofactors for many enzymes, K ions participate in many metabolic processes, such as nitrogen (N), sulfur (S), and phosphorus (P) assimilation, pyruvate synthesis, and sugar metabolism [52]. Potassium is taken up by the plant root through the epidermal and cortical cells, and then transported to the shoot and distributed to the leaves [53].

In general, there are two primary signaling pathways that play important roles in regulating K uptake, which include the CBL–CIPK network and ethylene-mediated pathways [54,55]. In the CBL–CIPK pathway, the Ca\(^{2+}\) sensor proteins (CBLs) interact with their target kinases (CIPKs). The balance of the cations was regulated by a number of transporting proteins that are involved in the K\(^{+}\) and Na\(^{+}\) uptake and translocation [31]. The report showed that both ethylene production and the transcription of genes of the ethylene biosynthesis increased when met with low potassium stress [56]. Reactive oxygen species (ROS) have been suggested to be upstream regulators of calcium signaling and participate in low potassium-signaling pathways [57,58]. In addition, ethylene acts upstream of the ROS in response to potassium deprivation by increasing the H\(_2\)O\(_2\) concentrations [55]. Therefore, it is necessary to clarify the molecular mechanisms of K ions’ uptake and transport in the CBL–CIPK network and ethylene-mediated pathways, especially in response to low −K\(^{+}\) conditions.

However, the regulation of potassium in plants is a complicated process that is coordinated with Ca, Na, N, and S ions [6,52]. As is well known, K\(^{+}\) absorption and translocation are mainly mediated by plant K\(^{+}\) transporters and channels [59]. At present, there are mainly three families of ion channels (Shaker, TPK/KCO, and TPC) and three families of transporters (HAK, HKT, and CPA) have been identified as contributing to K and Na ions transport across the plasmalemma and internal membranes [58,60]. Potassium transporters may function in both low-affinity and high-affinity transport as members of the KT/KUP/HAK family [31]. It has been reported that the protein kinase CIPK9 interacts with the calcium sensor CBL3 and plays an important role in K\(^{+}\) homeostasis under low-potassium stress in *Arabidopsis* [59]. In vivo and vitro experiments showed that the CBL1, CBL8, CBL9, and CBL10, together with a CBL-interacting serine/threonine protein kinase23 (CIPK23) activated a low K\(^{+}\) inducible potassium transporter HAK5 to regulate K\(^{+}\) transport in *Arabidopsis* [61]. In this data, we also identified 14 differentially expressed CBL-interacting protein kinases genes (Figures 8 and 9) in a low-potassium group of banana plants. Among these genes, two genes (*Ma06* *_g28510* and *Ma09* *_g04710*) were annotated to CBL-interacting serine/threonine protein kinases, which may play crucial roles in protein phosphorylation during low-potassium starvation.

Transcription factors (TFs) determine the temporal and spatial features of gene expression by binding to specific promoter sequences that comprise cis-regulatory elements [62]. Treated with low-potassium stress, significantly differentially expressed banana TFs about the MYB and MYB-related family genes were the largest subgroup of all the TFs in banana. It was reported that the AP2/ERF transcription factor RAP2.11 was a component in response to low potassium through regulation of the low-potassium signal transduction pathway via the high-affinity K\(^{+}\) uptake transporter AtHAK5, which was bound to a GCC-box of the AtHAK5 promoter [44]. At present, a total number of 112 DEGs about the AP2-EREBP transcription factors were identified, of which 25 of the DEGs were in banana leaves and 87 of the DEGs were in banana roots.

Transcriptome analysis is a common but excellent technological means to find genes that have participated in a variety of biotic or abiotic stresses in plants. Using RNA-Seq, this study provides important transcriptome information for banana (*Musa acuminata* L. AAA group, cv. Cavendish) in response to K\(^{+}\) starvation. The transcriptional profiles of banana genes have revealed that many genes are differentially regulated in leaves and roots after low potassium stress. Our data showed important genes that were related to K\(^{+}\) transport and uptake, including genes of the HAK/KUP/KT
system, AKT family, CBLs, and so on. These genes were differentially expressed between LK and NK treatments, especially in roots.

Here, we compared the mRNA expression levels of different banana tissues in low-potassium conditions. The expression patterns of mRNA among different tissues revealed that many important DEGs might regulate potassium expression at the transcriptional level. At present, transgenic research is the best method to clarify the functions of a gene. The successful acquisition of transgenic Cavendish bananas with resistance to fusarium wilt tropical race 4 (Foc4) brings a bright future for the study of genes response for K\(^+\) stress [63]. Many DEGs encoding protein kinases and ion transporters were simultaneously down/up-regulated in our data, suggesting that they may participate in the responses of banana to K\(^+\) deficiency. The data obtained in this study may enable a better understanding of the mechanisms controlling banana K\(^+\)-deficiency tolerance and will be useful in informing studies of K\(^+\) deficiency in other plant species. However, further research is required to validate the functions of the DEGs related to K\(^+\) stress.

5. Conclusions

The results of this study suggested that lots of DEGs related to K\(^+\) homeostasis participated in the mechanisms for low-potassium tolerance in banana. Although the genome of banana has been sequenced and assembled, genetic expression on the transcription level under low-potassium stress has not been reported in banana. This study main focused on analyzing the DEGs via the bioinformatics method and qRT-PCR assay. Our report provided a basic information for genes regarding transcription levels, and could be used to better understand the function of these genes under low-potassium tolerance.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/9/4/169/s1, Supplemental Material 1: The protocols of Hoagland complete culture solution and low-potassium (K\(^+\)) culture solution, Supplemental Material 2: Gene information for quantitative real-time polymerase chain reaction (qRT-PCR) validation.

Availability of Supporting Data: All genes ID number and genes sequence information are available in the Banana Genome Hub (http://banana--gonome-hub.southgreen.fr.). All supportive data and materials generated in this article will be made available on request. The raw data of high-throughput sequence (RNA-Seq) results and processed files have been submitted to GEO database of NCBI web site which accession number is GSE102968.

Author Contributions: H.T. conceived the study and participated in the design of all experiments. M.X. performed the qRT-PCR analysis, statistical analysis and drafted the manuscript. C.-B.Z., R.H., Z.Y. and Z.Q. prepared and watered the plant materials. R.X., Y.C. and S.-S.W. carried out the statistical analysis and participated in data analysis and discussion about the experiments. All authors read and approved the final manuscript.

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Conflicts of Interest: The authors declare that they have no conflict of interest.

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