

Transcriptome analysis and identification of the low potassium stress-responsive gene SiSnRK2.6 in foxtail millet (*Setaria italica* L.)

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Abstract

Foxtail millet (*Setaria italica* L.), which originated in China, has typical drought tolerance, barren tolerance, C₄ high light efficiency, strong adaptability, and high nutrient utilization characteristics. Nevertheless, the molecular mechanism of its tolerance to low-potassium stress is largely unclear. In this research, the low potassium tolerant variety “Yugu28” was screened out by low potassium stress treatment, and the transcriptome of “Yugu28” under low potassium was comprehensively analyzed. A total of 4254 differentially expressed genes (DEGs) were identified, including 1618 up-regulated and 2636 down-regulated genes, respectively. In addition, there were 302 transcription factor (TF) genes in the DEGs and MYB TFs accounted for the highest proportion, which was 14.9%. After functional analysis of all DEGs, a total of 7 genes directly related to potassium and 50 genes corresponding to hormones were screened. The expression levels of randomly selected 17 DEGs were verified by qRT-PCR and the results coincided well with the RNA-seq analysis, indicating the reliability of our transcriptome data. Moreover, one of the ABA signaling pathway genes, *SiSnRK2.6*, was identified and selected for further functional verification. Compared with the wild-type, transgenic rice with ecotopic expression of *SiSnRK2.6* showed remarkably increased root length and root number, indicating that overexpression of *SiSnRK2.6* can enhance the resistance of transgenic plants to low potassium stress.

Introduction

Potassium, which is known as the “quality element”, greatly affects plant growth and development. Previous literatures have shown that potassium deficiency is a key abiotic stress in plants (Yang et al. 2003; Cakmak et al. 2005). Functionally, potassium can be used as a nutrient or a signal substance to work in various metabolic pathways in the process of crop growth and development (Shaibur et al. 2008; Römheld et al. 2010). However, it was reported that 75% of the soil in China lack available potassium, leading to 8.39%-33.43% yield losses in millet (Du et al. 2019). Thus, improving low potassium stress tolerance of foxtail millet will ensure stable grain production.

Plants usually use their roots to absorb potassium from the soil and maintain normal life activities. However, when plants are exposed to low potassium stress, two important nutrient absorption mechanisms can be activated. One is the nutrient storage and distribution mechanism, which comprise some potassium channels and transporters (Ashley et al. 2006). Differently, potassium channels usually mediate the transport of low-affinity potassium transport proteins but the corresponding transporters mediate transportation of high-affinity potassium transport proteins (Cao et al. 2019). The other is the change in the growth and development processes of plant roots, such as taproot growth, lateral root formation, and so on (Lopez-Bucio et al. 2003). So far, two types of K⁺ transporters in *Arabidopsis thaliana* have been discovered, namely rectified K⁺ channel *AKT1* and low-potassium induced K⁺ transporter *HAK5* respectively, which mainly promote the absorption of K⁺ in plants under low-potassium stress (Gierth et al. 2005). The KT/HAK/KUP protein family is the earliest and most abundant potassium transporter protein family which is involved in the acquisition, redistribution, and homeostasis of

potassium and is important for plant growth, development, and resistance to abiotic stress (Li et al. 2018). HAK family members have been shown to play a crucial role in potassium absorption in several species, such as *HvHAK1* in barley (Santa-María et al. 1997), *Arabidopsis AtHAK5* (Gierth et al. 2005), and *OsHAK1* in rice (Bañuelos et al. 2002), which are associated with high root affinity for potassium absorption. In addition, *AtKUP7* (Han et al. 2016) in *Arabidopsis thaliana*, *OsHAK11* and *OsHAK12* (Okada et al. 2008) in rice, and *GhKEA4* and *GhKEA12* (Li et al. 2021) in upland cotton are strongly induced when potassium is deficient. Some HAKs encode low-affinity potassium transporters, such as *HvHAK2* in barley (Senn et al. 2001) and *CnHAK1* in seagrass (Garciadeblas et al. 2002). Under low potassium stress conditions, *OsHAK8* plays an important role in the absorption and transport of potassium ions (Wang et al. 2021). Moreover, the transcriptional regulation of diverse TFs also plays an important role in plant responses to low potassium stress (Wang et al. 2013; Chérel et al. 2014; Zhao et al. 2016). *SiMYB3* (Cao et al. 2019) and *SiNAC45* (Wang et al. 2015) were newly identified as two important regulatory genes related with low potassium stress in foxtail millet. Although some researchers aimed to uncover the function of plants responding to low potassium stress, the mechanisms underlying which in foxtail millet remain largely obscure.

Foxtail millet, as one of the oldest crops in the world, has typical drought-resistance and barren-insensitivity characteristics. In addition, millet possesses a small diploid genome (~ 515 Mb; 2N = 18) with great advantages in its short life cycle, self-pollination and prolific seed production (Bennetzen et al. 2012). Moreover, the completion of the genome sequencing has greatly promoted researches on the functional genomics of millet, which makes it an ideal model crop for studying the mechanism of plant stress resistance (Doust et al. 2009; Jia et al. 2013; Pandey et al. 2013; Muthamilarasan et al. 2014; Khan et al. 2014). However, few researches related with low potassium stress in millet are unveiled. Here, we report a comparative RNA-seq analysis to further explore the complex mechanism of low potassium tolerance in foxtail millet under potassium deficiency. Through low potassium stress treatment and phenotypic investigation of six millet varieties, we identified a low potassium tolerant variety “Yugu28” and used it to conduct a further transcriptome analysis. Through analyzing the transcriptome data, the DEGs responding to low potassium stress were screened, and the functional categorization of these genes was further performed. Additionally, one gene *SiSnRK2.6* was selected for functional verification, and we discovered that overexpression of *SiSnRK2.6* in rice can significantly improve the resistance of transgenic plants to low potassium stress. Our research laid the foundation for understanding the low potassium stress regulatory network in foxtail millet and also provided novel genetic resources for the improvement of low potassium stress tolerance in plants.

Materials and methods

Plant materials and growth conditions

In this study, the foxtail millet varieties were obtained from Professor Li Junxia of the Henan Academy of Agricultural Sciences. For low potassium stress treatment, seeds were initially sterilized and pregerminated on moistened filters for three days in an illumination incubator under the condition of 16h

light/8h dark (28°C/24°C) with 80% humidity. Then, the seedlings were transferred into a hydroponic box filled with the modified Hoagland nutrient solution (0.25 mM $\text{NH}_4\text{H}_2\text{PO}_4$, 1 mM CON_2H_4 , 2 mM KCl, 1 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.5 mM $\text{CaCl}_2 \cdot \text{H}_2\text{O}$, 0.1 mM Fe-EDTA, 1 μM H_3BO_3 , 0.05 μM $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, 0.5 μM $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 1 μM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 1 μM $\text{MnSO}_4 \cdot \text{H}_2\text{O}$). After three weeks of growth, half of the seedlings were further transferred to Hoagland nutrient solution with 3 μM KCl as the low potassium (LK) and the other half of the seedlings were still grown in the Hoagland nutrient solution with 2 mM KCl as a normal potassium treatment (NK). To ensure stable treatment, the Hoagland nutrient solution was replaced every two days. Eventually, phenotypic observation and sampling were performed after 10 days treatment.

Measurement of biomass and evaluation index

Three-week-old seedlings of different millet varieties growing under normal (NK) and low potassium (LK) conditions were weighted (shoots and roots separately) before (fresh weight) and after (dry weight) drying at 105°C for 30 min and 80°C for 48 h. The shoot-root ratio was defined as the value of the length of the shoot to that of the root. The relative index is calculated according to Cao et al. (2019). Five biological replicates were set for the fresh and dry weight measurements. The student's *t*-test was used for statistical significance detection.

RNA-seq analysis

The roots of three-week-old “Yugu28” seedlings under both control and low potassium stress conditions were detached and immediately frozen with liquid nitrogen. All samples were stored at -80°C until RNA extraction. Five independent seedlings were taken as one biological replicate, and three biological replicates were set for each control and experimental groups. Sample extraction and Illumina sequencing were performed by the Beijing Genome Institute (Hainan). Clean reads were mapped to the foxtail millet genome using *Phytozome* V13 (<https://phytozome-next.jgi.doe.gov/>).

Validation of the DEGs by quantitative real-time PCR

To ensure the reliability of the RNA-seq data, a quantitative real-time PCR was performed on the randomly selected 17 DEGs. qRT-PCR was performed using an ABI 7500 Real-time PCR system (Applied Biosystems). *SiActin7* was used as the internal control. Each experiment was performed with three biological replicates, and each biological replicate was assayed with three technical replications. The relative expression level was determined according to the $2^{-(\Delta\Delta\text{CT})}$ method (Livak et al. 2001).

Cloning of gene *SiSnRK2.6* and vector construction

To acquire the full-length coding sequence of gene *SiSnRK2.6*, total RNA was extracted from roots of the “Yugu1” variety using RNAiso Plus (Takara, Japan), and cDNA was obtained through a reverse transcription system (Takara, Japan). After getting the total cDNA, the coding sequence of *SiSnRK2.6* was amplified by the Primer Star DNA polymerase kit (Takara, Japan). Then, the obtained fragment was verified by agarose gel electrophoresis and cloned into pEASY-Blunt Zero vector (TransGene Biotech), and

the resulting constructs were verified by sequencing. Finally, the accurate coding sequence of *SiSnRK2.6* was ligated to the pCM1307 overexpression vector through a T₄ DNA ligase (Thermo Scientific).

Generation of transgenic rice plants and evaluation of the LK tolerance

To verify the function of gene *SiSnRK2.6* in low potassium stress tolerance, the *SiSnRK2.6*-pCM1307 overexpression vectors were transferred into the *japonica* rice variety Zhonghua11 (ZH11) by agrobacterium-mediated genetic transformation, and a series of *SiSnRK2.6* overexpression lines were obtained. T₂ transgenic plants were identified by qRT-PCR, and the positive transgenic plants were used for further phenotypic analysis. For phenotypic identification, the fully germinated seeds of the wild-type ZH11 and relevant *SiSnRK2.6* overexpression lines were firstly cultured with normal Hoagland nutrient solution under long-day growth conditions (28°C/24°C 16h light/8h dark) for 7 days. Then, the seedlings with consistent growth were transferred into a nutrient solution containing LK treatments for another 7 days. Finally, fifteen plants from each line were used for the measurements of root and shoot length, root number, fresh weight and dry weight.

Results

Phenotypic analysis of foxtail millet seedlings under K⁺ deficiency

In this study, low potassium stress treatment was carried out on different foxtail millet varieties. After 10 days treatment, the three-week-old seedlings showed decreased shoot and root growth for six different varieties (Table 1). Phenotypic analysis showed that the shoot-root ratio ranged from 1.21 to 2.78 among all the foxtail millet varieties, with “Yugu28” was 2.78, the highest among all varieties (Table 1). Consistently, there was no significant phenotypic difference for the “Yugu28” variety under low potassium treatment (Fig. 1 and Table 1). In addition, significant differences can be observed in the indices of shoot fresh weight and total dry weight among different varieties (Table 1). Especially for the relative index (X_{LK}/X_{NK}) of shoot fresh weight and total dry weight, which “Yugu28” reached the highest of all varieties, was 0.66 and 0.56, respectively (Table 1). Therefore, “Yugu28” was considered to be most tolerant to low potassium stress and was taken to conduct the transcriptome sequencing analysis.

Table 1

The ratio of shoot to root, shoot fresh weight and total dry weight of different varieties in the normal potassium (NK) and low potassium conditions (LK)

Variety	Shoot/Root		Shoot fresh weight			Total dry weight (g/plant)		
	NK	LK	NK	LK	Relative Index	NK	LK	Relative Index
Gonggu88	1.37c	1.21a	0.39b	0.22ab	0.55	0.07a	0.04b	0.53
Jigu22	1.97bc	1.54a	0.47ab	0.31a	0.65	0.09a	0.05b	0.55
An18h-6195	2.17abc	1.92a	0.48ab	0.23ab	0.49	0.12a	0.06ab	0.53
Yugu23	2.83a	2.11a	0.73a	0.21ab	0.28	0.16a	0.06b	0.38
An18-v5080	2.48ab	2.24a	0.38b	0.09b	0.23	0.1a	0.05b	0.49
Yugu28	2.43ab	2.78a	0.34b	0.22ab	0.66	0.16a	0.09a	0.56

Quality assessment of transcriptome sequencing output data

To make a global view of the transcriptome profile of the foxtail millet variety “Yugu28” under the low potassium treatment, high-throughput RNA-seq analyses were performed for the normal potassium (NK) and low potassium treatment (LK) libraries. After transcriptome sequencing, the original data and high-quality filtering data were analyzed. And it was found that the raw sequencing data ranged from 38.0 Mb to 72.8 Mb for each sample. After quality filtering, each sample produced 37.9 Mb to 72.8 Mb clean reads, and the comparison rate of these read lengths in the reference genome was more than 57.5%. The values of Q20 and Q30 in each sample were all above 90%. The above results indicated the high quality of RNA and transcriptome data, and laid the foundation for further analysis (Table S1).

Transcriptome analysis of “Yugu28” in response to low potassium stress

To verify whether variations in gene expression affect the phenotypic differences of foxtail millet in the case of potassium deficiency, we compared the differentially expressed genes (DEGs) between NK and LK samples. Transcriptome analysis revealed 4254 DEGs ($\log_2 \text{FC} \geq 1$ or $\log_2 \text{FC} \leq -1$) under low potassium stress treatment, including 1618 up-regulated and 2636 down-regulated genes (Fig. 2a, Table S2). In addition, the gene ontology (GO) analysis was performed to evaluate the potential functions of these DEGs by mapping each of them into the records of the GO database (<http://www.geneontology.org/>). Our results showed that these DEGs were mapped in different GO terms including biological processes (20 GO terms), cell components (11 GO terms), and molecular functions (9 GO terms) by three classification methods (Fig S1). Then the gene corresponding to the GO annotation

was enriched, and the significant enrichment pathway was screened according to the standard of q-value less than 0.05. The results showed that these genes were mainly enriched in 37 GO terms, among which 13 of the top 20 GO terms were distributed in molecular functions, 6 were distributed in biological processes, and only one was distributed in cell components (Fig. 2b, Table S3). Also, network construction was further carried out on the top 10 GO terms, and the results showed that the peroxidase activity (GO:0004601), oxidoreductase activity (GO:0016705) and heme binding (GO:0020037) in molecular function, response to oxidative stress (GO:0006979) and oxidation-reduction process (GO:0055114) in biological processes, were mainly clustered into one category; carbohydrate metabolic process (GO:0005975) in biological processes and hydrolase activity (GO:0004553) in molecular function, were mainly clustered into one category; protein kinase activity (GO:0004672) in molecular function and protein phosphorylation (GO:0006468) in biological process were clustered into one category; whereas, transporter activity (GO:0005215) in molecular function was grouped into a separate category (Fig S2, Table S3).

As it is known that transcriptional regulation mediated by diverse TFs usually plays an important role in plant stress response, thus the TFs in the DEGs under low-potassium stress treatment were taken for further analysis. A total of 302 TFs (108 up-regulated and 194 down-regulated, respectively) were found in “Yugu28” after low potassium stress treatment (Fig. 3, Table S4). These 302 TF genes originated from 41 different gene families, mainly including 45 MYB (8 up-regulated and 37 down-regulated, respectively), 33 bHLH (19 up-regulated and 14 down-regulated, respectively), 32 WRKY (11 up-regulated and 21 down-regulated, respectively), 24 NAC (6 up-regulated and 18 down-regulated, respectively), 19 bZIP (6 up-regulated and 13 down-regulated, respectively), 17 HD-ZIP (5 up-regulated and 12 down-regulated, respectively), and 131 others (53 up-regulated and 79 down-regulated, respectively) (Fig. 3, Table S4). Among these families, the largest number of differentially expressed TFs was in the MYB TF family (14.9%), followed by the bHLH TF family (10.9%), the WRKY TF family (10.6%) and the NAC TF family (7.9%).

KEGG analyses underlying low potassium stress

To identify the metabolic pathways in which these DEGs are involved, a KEGG analysis was further performed. Our results showed that 963 DEGs were enriched in a total of 117 pathways and the top 20 pathways were displayed in Fig. 4. We noticed that most DEGs were involved in seven main pathways, including phenylpropanoid biosynthesis (88 genes), glutathione metabolism (43 genes), amino sugar and nucleotide sugar metabolism (37 genes), starch and sucrose metabolism (33 genes), cysteine and methionine metabolism (24 genes), cyanoamino acid metabolism (20 genes) and the MAPK signaling pathway (20 genes) (Fig S3, Table S5).

Functional analysis of DEGs responding to low potassium stress

It has been reported that plants can adapt to the low potassium stress condition by enhancing their own potassium absorption efficiency or adjusting the redistribution and utilization of endogenous potassium, which is mainly accomplished by two potassium transport systems: potassium transporters and potassium ion channels (Maathuis et al., 1997). Therefore, in this study, differentially expressed genes directly related to potassium under low potassium stress were screened, and a total of 7 genes were identified (Table 2). There were 6 potassium transporter genes (*Seita.9G314300*, *Seita.2G224400*, *Seita.9G412000*, *Seita.7G082700*, *Seita.9G182300*, and *Seita.5G439500*) and 1 potassium ion binding protein gene (*Seita.9G182400*). Among these 7 DEGs, five genes (*Seita.9G314300*, *Seita.2G224400*, *Seita.9G412000*, *Seita.7G082700*, and *Seita.9G182300*) were up-regulated, and two genes (*Seita.5G439500* and *Seita.9G182400*) were down-regulated under low potassium stress, suggesting that these genes may play an important role in low potassium stress response.

In addition, due to the extensive involvement of phytohormones in the physiological and biochemical responses to environmental stress, we further analyzed phytohormone-related DEGs from the transcriptome data. A total of 50 DEGs (16 up-regulated and 34 down-regulated, respectively) were discovered to encode proteins which were annotated to hormone response-related pathways, including 31 auxin response proteins, 9 ethylene response proteins, 6 gibberellin-related proteins, 2 brassinosteroid-related proteins, 1 ABA response protein, and 1 jasmonic acid response protein (Table S6). Further analysis showed that the auxin pathway mainly contained 18 *AUX/IAA*, 8 *SAUR*, and 5 *GH3* genes, which regulated the growth of millet plants by changing their expression levels when foxtail millet was imposed upon low potassium stress. In addition, there are 9 genes in the ethylene synthesis pathway, including 1 *EIN3* (down regulated by 1.64 times), 5 *ERF* (one up regulated by 1.84 times and four down regulated by 1 ~ 2.25 times), and 3 *EFE* (down regulated by 4.72, 2.77, and 3.21 times). These pathway genes laid the foundation for revealing the molecular mechanism about how plant hormones respond to low potassium stress. A possible explanation is that low potassium stress may induce the differential expression of these genes and regulate hormone synthesis, thus affecting the growth and development of millet.

Table 2
Screening of genes directly related to potassium under low potassium stress in foxtail millet

Gene ID	Gene name	log ₂ FC	Annotation
<i>Seita.9G314300</i>	plastidic pyruvate kinase beta subunit 1	1.41	Potassium transporter
<i>Seita.2G224400</i>	high affinity K ⁺ transporter 5	1.58	Potassium transporter
<i>Seita.9G412000</i>	potassium transporter 2	1.81	Potassium transporter
<i>Seita.7G082700</i>	high affinity K ⁺ transporter 5	2.78	Potassium transporter
<i>Seita.9G182300</i>	high affinity K ⁺ transporter 5	1.35	Potassium transporter
<i>Seita.5G439500</i>	high affinity K ⁺ transporter 5	-1.95	Potassium transporter
<i>Seita.9G182400</i>	high affinity K ⁺ transporter 5	-8.26	Potassium ion binding

qRT-PCR validation and the expression level of gene *SiSnRK2.6* in rice transgenic lines

In order to verify the reliability of transcriptome data, 8 up-regulated and 9 down-regulated genes were randomly selected for qRT-PCR analyses (Table S7). The results showed that these 17 DEGs exhibited similar expression patterns as shown in the transcriptome analysis (Fig. 5), indicating that the results of RNA-seq were reliable. Among all the DEGs, we found that the expression of gene *SiSnRK2.6* (gene ID: *Seita.9G318200*) was highly up-regulated (Table S2), suggesting a possible function of *SiSnRK2.6* in regulating low potassium stress. To confirm our hypothesis, we overexpressed *SiSnRK2.6* in rice plants ZH11 and generated a series of transgenic lines. Moreover, our results showed that the expression level of *SiSnRK2.6* was significantly elevated in these overexpression lines compared with that of the wild type (Fig S4).

Overexpressing *SiSnRK2.6* in rice shows significantly enhanced low potassium stress tolerance

To further confirm the role of *SiSnRK2.6* in low potassium stress, different overexpression lines (OE-1, OE-2 and OE-3) and the wild-type rice plants were conducted with normal and low potassium treatments. Phenotypic analysis showed that no significant difference could be observed between overexpression lines and the wild-type seedlings under normal growth conditions (Fig. 6). However, when they were conducted low potassium stress treatment, their growth was both inhibited but with significantly weaker degrees of *SiSnRK2.6* overexpression lines than that of the wild-type (Fig. 6a). Further statistical analysis showed that overexpression lines presented significantly higher root and shoot length (Fig. 6b, c), root number (Fig. 6d), fresh weight (Fig. 6e) and dry weight (Fig. 6f) than those of the wild-type under low potassium stress treatment. These results showed that *SiSnRK2.6*-overexpression plants may respond to low potassium stress by increasing root length and root number to maintain their normal growth and promoting potassium absorption, indicating that overexpressing *SiSnRK2.6* could improve K⁺ deficiency tolerance at the seedling stage. Therefore, *SiSnRK2.6* is a promising target for improving low potassium stress tolerance in plants.

Discussion

Foxtail millet [*Setaria italica* (L.) Beauv.], which originated and has been domesticated in China for about 8700 years (He et al. 2023). Millet can achieve satisfactory yield levels under low fertility conditions by enhancing the accumulation of biomass (Nadeem et al. 2018). Though few studies revealed the resistance of millet to low potassium stress at the transcriptional level (Cao et al. 2019), the detail regulatory mechanisms of foxtail millet tolerance to low potassium stress remain largely unclear. In this study, the DEGs of millet under low potassium stress condition were comprehensively analyzed by using the RNA-seq approach, and a number of candidate genes responding to low potassium stress were screened, which provided a theoretical basis and important gene resources for further understanding the mechanism of foxtail millet responding to low potassium stress at the seedling stage. In the previous research, Cao et al. (2019) used the GO and KEGG analysis to analyze the expression profiles, classification characteristics, and clustering pathways of DEGs of “Longgu25”, under normal and low

potassium treatments. In this study, DEGs were screened under NK and LK conditions, and a total of 4254 DEGs were screened (Fig. 2a, Table S2). Compared with the DEGs screened under low potassium stress in “Longgu25”, a low potassium stress-tolerant variety, we found only 463 common DEGs. These genes shared by different varieties are likely to be the key targets regulating the low potassium stress tolerance of foxtail millet, so the functional verification and utilization of these genes can be an effective method for breeding new foxtail millet varieties with low potassium stress tolerance. Consequently, these genes can be the focus of further research.

For plants, response to low potassium stress may involve a variety of biological processes. In our study, KEGG analysis showed that these DEGs were mainly enriched in some biological pathways related to molecular function, such as binding, transporter activity, and so on (Fig. 2b, Table S3). In the previous report, transcription factors have been discovered to play an important role in the processes of plant abiotic stress response (Singh et al. 2002). In the present study, a total of 302 differentially expressed transcription factors were found (Fig. 3, Table S4). These TF genes were from 41 different gene families, and the MYB TF family comprises the largest number of DEGs (~ 14.9%). In addition, many studies have shown that MYB TFs play important roles in regulating low temperature and drought resistance, such as *OsMYB3R-2* (Ma et al. 2009), *OsMYB4* (Pasquali et al. 2008) and *AtMYB15* (Ding et al. 2009; Agarwal et al. 2006). However, only few studies uncovered the function of MYB TFs in regulating low potassium stress (Cao et al. 2019). Therefore, further researches are required to reveal the mechanisms of MYB TFs underlying potassium deficiency.

When encountered low potassium stress, plants often adjust the expression of potassium-related genes to activate their own potassium absorption and utilization system or potassium redistribution and utilization system to cope with potassium deficiency environment (Maathuis et al. 1997). Similarly, plant hormones also play an integral role in plant abiotic stress responses (Waadt et al. 2022). Thus, we analyzed the DEGs directly related to potassium and hormone-related genes in this study, and a total of seven genes related to potassium and fifty genes related to hormones were found (Table 2 and Table S6). Large numbers of DEGs were discovered under low potassium stress, and the expression of AKT channel protein in flax was significantly up-regulated after low potassium treatment (Huang et al. 2021), while *CIPK23* and *KC1* co-regulated *AKT1* to respond to low potassium stress in *Arabidopsis thaliana* (Wang et al. 2016). Overexpression of *AKT1* in rice can improve its resistance to osmotic and drought stress through increasing the content of K^+ in tissues (Ahmad et al. 2016), indicating that AKT channel protein has similar functions in millet, flax, *Arabidopsis* and rice. Since the expression levels of these genes are obviously changed in millet under low potassium stress, it is speculated that these genes may play an important role in response to low potassium stress.

Absciscic acid (ABA) has been reported as a stress response hormone (Cutler et al. 2010). In the current study, we found that the expression of *SiSnRK2.6*, an ABA signaling related gene, was significantly up-regulated under low potassium stress (Table S2). In addition, our genetic evidence showed that overexpression of *SiSnRK2.6* in rice significantly increased low potassium stress resistance compared with the wild type ZH11 (Fig. 6). *SnRK2.6* was previously identified as a crucial gene for regulating ABA-

mediated stomatal aperture in *Arabidopsis* (Mustilli et al. 2002; Yoshida et al. 2002). In this study, we revealed the role of *SiSnRK2.6* in modulating low potassium stress, which was a functional extension of SnRK-related genes, and further researches are still needed on the tolerance mechanism of *SiSnRK2.6* to low potassium stress. In summary, we conducted a genome-wide transcriptomic analysis of foxtail millet under low potassium stress treatment, and the results lay the foundation for elucidating the molecular mechanism of foxtail millet in response to low potassium stress and contribute to the practical application of tolerant genes in the breeding.

Declarations

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Author contributions All authors contributed to the study conception and design. Xiaoqian Ma and Junxia Li designed the research. Material preparation, data collection and analysis were performed by Xiaoqian Ma, Shutao Dai and Na Qin. Zanning Han and Bing Guo provided technical assistance to Xiaoqian Ma. The first draft of the manuscript was written by Xiaoqian Ma, Najeeb Ullah Khan conceived and supervised the writing and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability All data supporting the conclusions of this article are provided within the article and its Supplementary Information.

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Figures

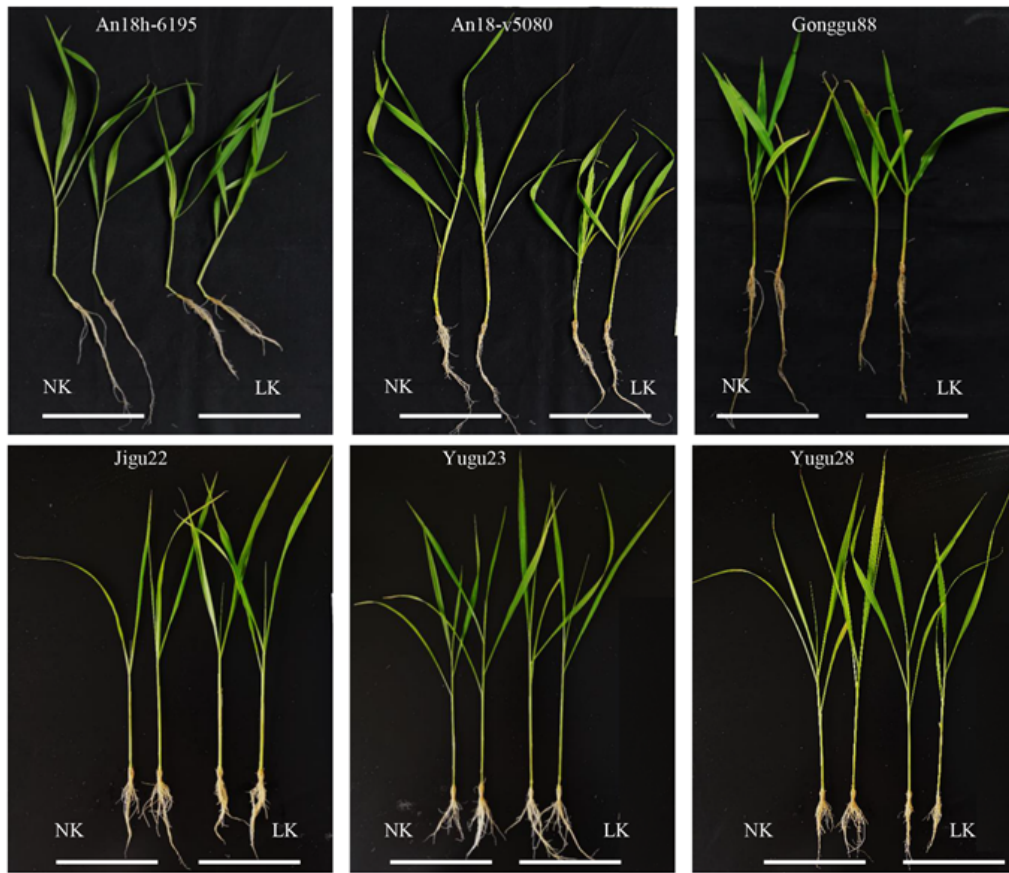


Fig.1 Images of six foxtail millet varieties growing under normal and low potassium conditions at seedling stage. The Hoagland nutrient solution containing 3 μ M KCl are treated as low potassium (LK). The plants cultivated in normal potassium (NK) are shown on the left and seedlings treated with low potassium (LK) are shown on the right.

See image above for figure legend

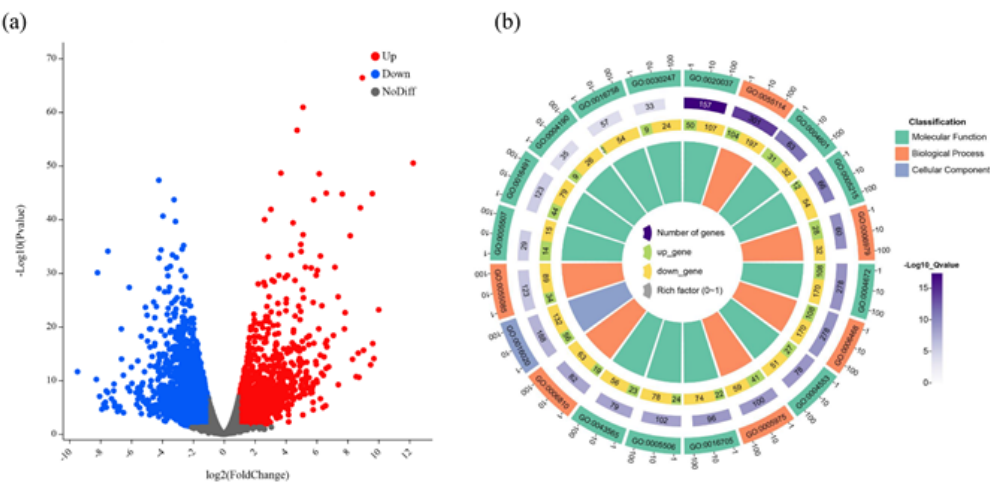


Fig.2 Gene Ontology (GO) enrichment analysis of differentially expressed genes (DEGs). (a) Volcano map of DEGs between the normal potassium (NK) and low potassium group (LK). (b) The top 20 GO terms of the gene corresponding to the GO annotation, including 13 categories in molecular function, 6 categories in biological process and 1 category in cellular component.

Figure 2

See image above for figure legend

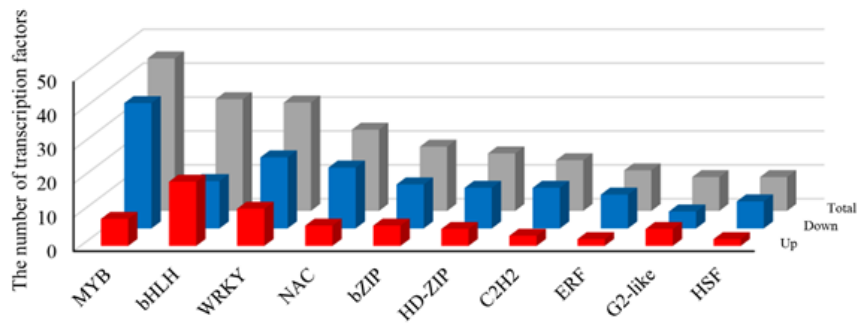


Fig.3 Distribution graphs of differentially expressed genes (DEGs) which belong to transcription factor gene families after low potassium stress treatment.

Figure 3

See image above for figure legend

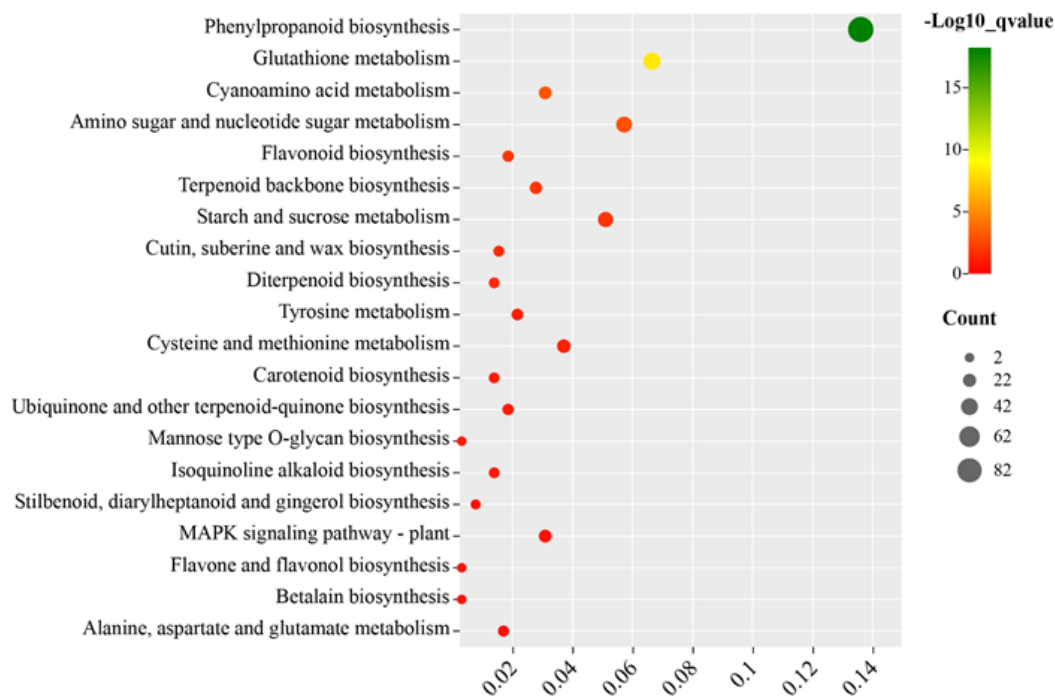


Fig.4 Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of the DEGs. The top 20 main pathways underlying low potassium stress were displayed.

Figure 4

See image above for figure legend

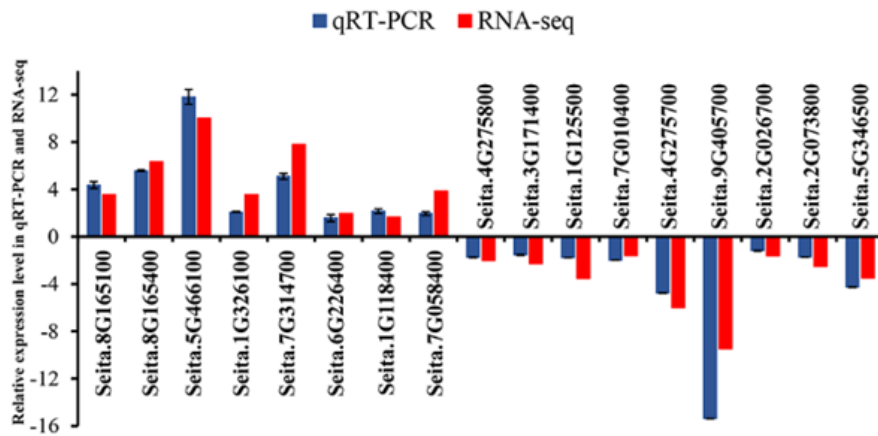


Fig.5 Validation of differentially expressed genes by qRT-PCR. To confirm the reliability of Illumina sequencing technology, 17 candidate genes were randomly selected and their expression levels were confirmed by qRT-PCR.

Figure 5

See image above for figure legend

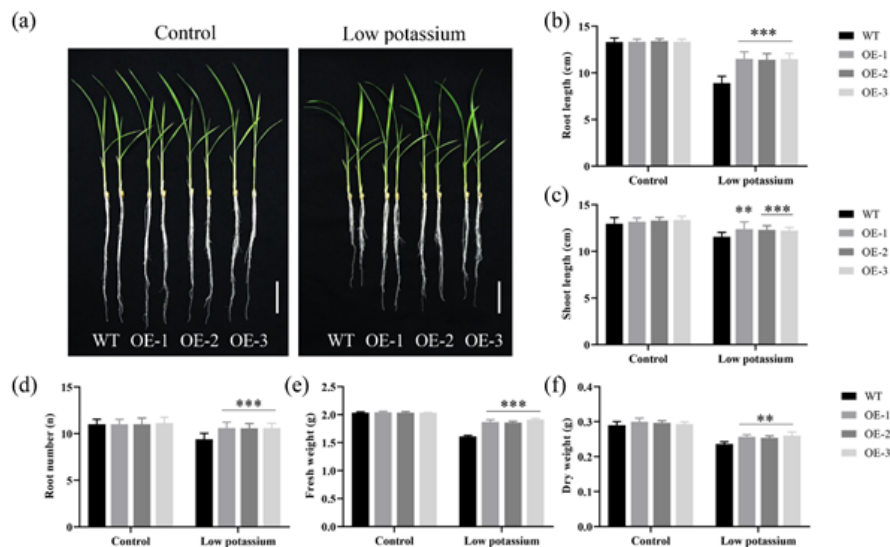


Fig.6 Phenotypic identification of *SiSnRK2.6* overexpression seedlings after growing under normal potassium or low potassium stress conditions. Images of the wild-type and *SiSnRK2.6* overexpression lines (a) and investigation of the root length (b), shoot length (c), root number (d), fresh weight (e) and dry weight (f) under control and low potassium stress treatment, respectively. Data are means $\pm$ SD ($n = 15$). Statistical significance was determined by the student's *t*-test. ** and *** indicate $p < 0.01$ and $p < 0.001$, respectively.

Figure 6

See image above for figure legend

Supplementary Files

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