Transcriptional and Metabolic Responses of Maize Shoots to Long-Term Potassium Deficiency

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Abstract

Aims

Potassium is important for plant growth and crop yield. However, the effects of potassium (K^+) deficiency on silage maize biomass yield and how maize shoot feedback mechanisms of K^+ deficiency regulating whole plant growth remains largely unknown. Here, the study aims to explore the maize growth and transcriptional and metabolic responses of shoots to long-term potassium deficiency.

Methods

The growth of silage maize and its biomass were analyzed with K^+ treatment in field and hydroponic experiments. Furthermore, transcriptional and metabolic profiles of shoots were investigated for their effects on maize development under K^+ deficiency condition.

Results

Under K^+ insufficiency condition, the biomass yield of silage maize decreased by 14%-17% in two-year field trials. The transcriptome data showed that there were 390 differently expressed genes overlapping and similarly regulated in the two varieties and they were considered as the fundamental responses to K^+ deficiency in maize shoots, with many stress-induced genes involved in transport, primary and secondary metabolism, regulation, and other processes involved in K^+ acquisition and homeostasis. Metabolic profiles indicated that most amino acids, phenolic acids, organic acids, and alkaloids were accumulated in shoots under K^+ deficiency condition and part of the sugars and sugar alcohols also increased.

Conclusion

Our results suggested putrescine and putrescine derivatives were specifically accumulated under K^+ deficiency condition, which may play a role in feedback regulation of shoot growth. These results confirmed the importance of K^+ on silage maize production and provided a deeper insight into the responses to K^+ deficiency in maize shoots.

Introduction

Potassium (K^+) is one of the essential macronutrients required for plant growth and development, such as photosynthesis, osmoregulation, enzyme activation, protein synthesis, and ion homeostasis (Hafsi et al. 2014; Kanai et al. 2011). A large area of cropland has low levels of K^+ , and crops cannot efficiently utilize the mineral elements in the soil (Hafsi et al. 2014; Perry et al. 1972). Applying K^+ fertilizer can increase the crop yield, but the intensified use of K^+ fertilizer in agriculture gives rise to environmental pollution (Römheld and Kirkby 2010). From the year 1961 to 2015, the estimated K^+ utilization efficiency of cereal crop was only 19%, underscoring the need to conserve this non-renewable natural resource

(Dhillon et al. 2019). Therefore, improving K^+ utilization efficiency of crop plants is crucial for optimizing fertilizer use, improving crop yield, and reducing environmental pollution. To optimize the K^+ utilization of specific crop species, it is important to investigate the response and adaptations to K^+ deficiency and its underlying mechanisms.

The potassium is absorbed by plant roots and transported to the aboveground tissues, in which K^+ transporters and channels play important roles in absorbing and transporting potassium (Jia et al. 2008). Under K^+ deficiency condition, plants change their root structure and modify their root hairs to absorb more nutrients (Jia et al. 2008). Recently, the transcriptome profiles of plant roots under K^+ deficiency condition have been analyzed in rice, wheat, soybean, cotton, and tomato (Ma et al. 2012; Ruan et al. 2015; Singh and Reddy 2017; Yang et al. 2021; Zhao et al. 2018). Genes involved in metabolic pathways such as carbohydrates, plant hormones, and kinases play indispensable roles in maintaining the plant growth under K^+ deficiency condition, as well as transcription factors (Hyun et al. 2014; Yang et al. 2021; Zhao et al. 2018). Moreover, shoot can feedback on regulating the uptake of mineral nutrients (such as nitrogen and phosphate) from roots (Bari et al. 2006; Tabata et al. 2014). Regulators, such as high-affinity K^+ transporter, were identified to be associated with shoot regulation of root K^+ uptake in response to its deficiency in cotton (Wang et al. 2019b). Thus, it is important to analyze the adaptation of plant shoot under K^+ deficiency as well.

Maize (*Zea mays* L.) is a multi-functional important crop in food, animal feed and energy production (Schnable 2012). Besides, maize can be used as silage maize, which is an important feed for intense ruminant production, exhibiting high biomass production and relatively low input demand (Baghdadi et al. 2018). The growth of maize relies heavily on the use of chemical fertilizers, besides potassium (Baghdadi et al. 2018). Potassium deficiency is an abiotic stress of crops, which restricts their yield (Qin et al. 2019; Zorb et al. 2014). Plants would response to potassium deficiency in different levels (Hafsi et al. 2014). Low potassium level would induce lateral root growth in maize, in which genes associated with nutrient utilization, hormones and transcription factors are involved (Ma et al. 2020; Zhao et al. 2016). Although the root system underlies potassium absorption, it is unclear how low K^+ concentration induces the expression of related genes and metabolites in maize shoots to maintain its shoot growth and promote root absorption to accommodate K^+ deficiency.

In this study, the growth of silage maize and its biomass were analyzed with K^+ treatment in field and hydroponic experiments. Furthermore, transcriptional and metabolic profiles of shoots were investigated for their effects on maize development under K^+ deficiency condition. The study was to determine the primary effects of K^+ deficiency on the growth of silage maize, and to explore the potential transcriptional and metabolic responses in shoots. This study helps to understand the physiological adaptation mechanisms of corn silage development under K^+ deficiency condition and provides a theoretical basis for improving the nutrient utilization efficiency of silage maize in breeding.

Materials And Methods

Field trial and trait evaluation

The field experiment lasted for two years to assess the effects of potassium on silage maize biomass production from 2019 to 2020 at a cropland (36°26' N, 120°5' E) of Qingdao, Shandong Province, China. The plowed soil (0-20 cm) contained 9.16 g/kg organic matter, 0.92 g/kg total N, 33.25 mg/kg available P, and 70.67 mg/kg available K. Maize was sowed in mid-June and harvested in late September, following normal agricultural practices. Hybrid DH605 was cultivated and plots were arranged in 6 rows (60 cm wide) and 5 m in length, with a density of 67,500 plants per hectare. Maize was fertilized with a uniform dose of 315 kg N and 90 kg P₂O₅ per hectare. Full dose of P and K and 2/3 of N was band applied at sowing and the remaining 1/3 of N was applied at V10-V12 stage. For potassium treatment, one group (K0) was treated with no additional K₂O application and the other group (K1) applied with K₂O 40.5 kg per hectare. Three trials were conducted for each treatment.

The maize was harvested about 15 cm off the ground with an area of 1.8 m × 5 m per plot at approximately half starch milk line stage for biomass yield calculation. The plant height was measured from ground to tassel. The third stem node from the bottom was measured as the stem diameter. The average values of the ten plants were calculated from each replicate.

Plant growth and sample collection for hydroponic experiment

For hydroponic experiment, maize seeds were surface sterilized in 10% H₂O₂ for 20 min, rinsed and then germinated in coarse quartz sand until two leaves were visible. The seedlings were grown in the chamber for three days (16 h light/8 h dark, 25°C, 60% relative humidity), cultured with modified Hoagland solution (Zhao et al. 2012). The seedlings were transferred to a nutrient treatment solution containing either 0.1 mM KCl (K⁺ deficiency; LK) or 4 mM KCl (K⁺ sufficiency; CK) to assess their response. The solution was refreshed every three days and the treatment lasted for 12 days. There were three biological replicates. Plant shoots used for K⁺ concentration, metabolic and transcriptional analysis were immediately frozen in liquid nitrogen and then stored at -80°C until use.

Photosynthetic rate in leaves and measurement of soluble K⁺ concentration

The photosynthetic rate of the uppermost newly and fully expanded leaves was measured with an LI-6800 portable photosynthesis system (LI-COR Inc., Lincoln, NE, USA) after 12 days treatment. The photosynthetically active radiation was set to 800 μmol m⁻² sec⁻¹. The freeze-dried and powered shoot samples (0.2 g) were used to analyze the K⁺ concentration using a flame photometer (Model 410, Sherwood Scientific Ltd, Cambridge, UK) following the previously described H₂SO₄-H₂O₂ decoction method (Kanai et al. 2007).

RNA isolation, qPCR and transcriptome analysis

Total RNA was isolated using Trizol reagent (Invitrogen, Carlsbad, CA, USA) and RNA quality and integrity was determined using Nanodrop 2000 (Thermo, Waltham, Massachusetts, USA). For RNA sequencing

(RNA-seq) library synthesis, three biological replicates per treatment were sequenced with an Illumina HiSeq-PE150 instrument. Gene expression levels were normalized by calculating reads per kilo base of transcript per million fragments mapped (RPKM) using HISAT2 2.0.5 after mapping to the B73 reference genome sequences (Zm-B73-REFERENCE-NAM-5.0;

https://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/902/167/145/GCF_902167145.1_Zm-B73-REFERENCE-NAM-5.0/). The absolute value of $\log_2$ (fold change) ≥ 1 (treatment/control) ($p \leq 0.05$) was defined as differentially expressed gene transcripts (DEGs). DEGs were also employed for Gene Ontology (Go) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis.

Metabolite measurements

To analyze the metabolite under K^+ deficiency, metabolite abundances were extracted and quantified at Wuhan MetWare Biotechnology Co., Ltd. (www.metware.cn) (Wang et al. 2019a). The maize shoots were freeze-dried, powdered and then extracted with 70% v/v aqueous methanol at 4°C. The extractions were centrifuged at 12 000 g for 10 min, and then filtered (0.22 μ m pore size). The obtained supernatants were analyzed using an UPLC-MS/MS system (UPLC, SHIMADZU Nexera X2; MS/MS, Applied Biosystems 4500 QTRAP). Principal component analysis (PCA) was used to analyze the differential accumulated metabolites (DAMs) between samples of K^+ treatments. The relative importance of metabolites was checked with the parameter of variable importance in project (VIP). Metabolites with $VIP \geq 1$ and an absolute value of $\log_2$ (fold change) ≥ 1 (treatment/control) were considered as DAM.

Data analysis

Data on plant stem diameter, fresh weight, yield, K^+ concentration and photosynthetic rate were examined using SPSS software. Significance was defined as a probability level of the student's t test at $p \leq 0.05$.

Results

Effect of applying potassium fertilizer on silage maize field biomass yield

Potassium is one of three essential macronutrients required for crop growth. From field trials, the maize plant height and stem diameter were significantly reduced without additional potassium fertilizer application (Table S1). The fresh weight per plant without applying additional potassium fertilizer was dramatically lower than maize supplying potassium fertilizer, thus significantly decreased the silage maize biomass (Table S1). With potassium fertilizer application in 2019 and 2020, the biomass yield increased by 17% and 14%, respectively (Fig. 1).

Effect of K^+ deficiency on maize growth

To investigate the mechanism of K⁺ deficiency for maize growth, two varieties (DH605 and Z58) were used to investigate their responses to K⁺ deficiency (0.1 mM) in hydroponic experiment. The results showed that K⁺ deficiency significantly decreased the shoot height and root length of both varieties and their stems were slender (Fig. 2A-D). Moreover, the fresh weight of shoots significantly decreased by 57.5% and 65.5% in DH605 and Z58 under K⁺ deficiency condition and the root fresh weight also showed a similar trend (Fig. 2E). The K⁺ deficiency in the hydroponic solution resulted in a larger reduction in K⁺ content of shoots, with the K⁺ concentration reduced by 80.16% and 83.54% in DH605 and Z58, respectively (Fig. 3A). Under K⁺ deficiency condition, maize net photosynthetic rate displayed a dramatic reduction in both varieties compared to plants grown under K⁺ sufficiency condition (Fig. 3B).

Global transcriptional changes in response to K⁺ deficiency

To obtain a global overview of the transcriptome relevant to K⁺ deficiency in maize, RNA-seq libraries from shoot samples of DH605 and Z58 were set up after 12 days treatment under K⁺ deficiency condition. After the removal of low-quality reads, an average of 4.5×10^7 clean reads were obtained for each sample, and the total length of the clean reads reached above 6.2×10^9 nt. For each sample, 97.5% of the clean reads were mapped to the maize reference transcriptome (Table S2). The DEGs were 922 in DH605, including 676 upregulated and 246 downregulated genes (Fig. 3C; Table S3a). 1106 DEGs were detected in Z58, including 922 upregulated and 184 downregulated genes (Fig. 3C; Table S3b). Moreover, 352 genes were upregulated in both DH605 and Z58, and the number of co-downregulated genes was only 38 (Fig. 3C). Among the co-regulated DEGs, the expression of specific K⁺ transporters related genes were indeed induced in a K⁺ deficient environment (Table 1). The expression levels of high-affinity K⁺ transporter (*HAK*) genes (*LOC100502520-HAK1* and *LOC100384472-HAK5*) were up-regulated under K⁺ deficiency condition. Gene encoding inward potassium channels (*LOC100281406-AKT2*) was also up-regulated to promote uptake of K⁺ both in maize shoots. Moreover, ten genes encoding ABC transporters were also up-regulated for both two varieties, which are reported to be involved in transport mineral and organic ions, amino acids, oligosaccharide, lipids, and metal ions (Table 1) (Xie et al. 2020).

Table 1

Genes encoding transporters showed differential expression in response to K⁺ deficiency (0.1 mM). The value indicates increase or decrease in log₂ (fold change) ≥ 1 (treatment/control).

Gene family	Seq ID	DH605	Z58	Gene description
ABC transporter	<i>LOC100274125</i>	1.74	1.36	ABC transporter C family member 3
	<i>LOC100286314</i>	2.67	3.17	ABC transporter B family member 2-like
	<i>LOC100381832</i>	2.01	1.55	ABC transporter C family member 4
	<i>LOC100384044</i>	1.97	2.85	ABC transporter G family member 36
	<i>LOC100501068</i>	2.23	1.38	ABC transporter G family member 9-like
	<i>LOC103633239</i>	2.19	2.55	ABC transporter G family member 43
	<i>LOC103636202</i>	2.46	3.66	ABC transporter B family member 11
	<i>LOC103641937</i>	2.90	3.76	ABC transporter C family member 15
	<i>LOC103652531</i>	2.52	1.91	ABC transporter B family member 9
	<i>LOC103653413</i>	5.22	5.11	ABC transporter A family member 7
AKT	<i>LOC100281406</i>	2.36	1.92	potassium channel AKT2
HAK/KT/KUP	<i>LOC100502520</i>	1.61	1.51	potassium high-affinity transporter HAK1
	<i>LOC100384472</i>	4.38	4.97	potassium high-affinity transporter HAK5
	<i>LOC100281081</i>	-1.82	-1.34	potassium transporter HAK10
	<i>LOC103645946</i>	-1.72	-1.79	potassium transporter HAK11

To gain a general understanding of the responses to K⁺ deficiency, GO and KEGG pathway enrichment analyses were performed (Fig. 4; Table S4). Among the induced pathways under K⁺ deficiency condition, the top 15 pathways of significant enrichment are shown in Figure 4C. Most of the pathways were co-regulated in DH605 and Z58, associated primarily with regulatory processes, transport, primary and secondary metabolism, including “ABC transporters”, “MAPK signaling pathway”, “Plant hormone signaling transduction”, “amino sugar and nucleotide sugar metabolism”, “biosynthesis of amino acids”, “starch and sucrose metabolism”, “glutathione metabolism”, and “glycerophospholipid metabolism” (Fig. 4).

Transcription factors (TFs) also play pivotal roles in regulating related genes in response to stress in plants (Ulm et al. 2004). In this study, 38 transcription factors were identified for both DH605 and Z58 (Table S5). These TFs belonged to diverse families, including AP2/ERF (2), bZIP (2), WRKY (9), MYB and MYB-related (5), NAC (3), bHLH (3), zinc finger (2), PLATZ (3), HB-HD-ZIP (2), GARP-G2-like (1), SRS (1), Tify (1), HSF (1), LOB (1), GNAT (1), MBF1(1). Among them, the number of WRKY genes accounted for 23.6% of the total regulated TFs.

Metabolic responses to K⁺ deficiency treatment

Primary metabolites were profiled by UPLC-MS/MS to gain insight into the possible molecular metabolic mechanism of maize under K⁺ deficiency treatment. Principal component analysis (PCA) was conducted on a total of 273 and 120 differential accumulated metabolites (DAMs) in DH605 and Z58, respectively (Table S6). The PCA results showed a trend of separation among the groups (Fig. S1).

Profiled metabolites can be classified into seven categories, including amino acids, organic acids, phenolic acids, nucleotides and derivatives, sugars and sugar alcohols, alkaloids, and lipids. In general, levels of most amino acids, alkaloids, phenolic acids and nucleotides and derivatives increased with K⁺ stress in both DH605 and Z58 (Table 2; Table S6). The accumulated amino acids in both varieties including Ser, Val, Asn, Thr, and homoserine, as well as the non-proteinogenic amino acids such as γ -amino-butyric acid (GABA). The saccharide, like raffinose, also accumulated in shoots. Carbohydrates, such as galactinol, sucrose, maltose, and trehalose, were decreased in DH605 shoots (Table S6a). Part of the organic acids level (L-homoserine, 4-guanidinobutyric acid, citric acid, and isocitrate) increased, while organic acid like methylene succinic acid and shikimic acid decreased in both varieties. The lipids decreased with K⁺ stress. Sugars and sugar alcohols levels, such as turanose, nicotinate D-ribonucleoside, D-glucosamine and raffinose increased under K⁺ stress in both varieties, while gluconic acid and D-saccharic acid decreased. Other sugars and sugar alcohols showed a variation under K⁺ deficiency treatment of DH605 and Z58 shoots.

Table 2 Heat-map of metabolite profiles. The value indicates metabolite changes in log2 (fold change) ≥ 1 (treatment/control). Shades of red or green indicate increase or decrease in Log2FC value, respectively. Values with log2FC ≥ 1 or ≤ -1 but afflicted with a high t test are highlighted with different background colors.

Group	Compounds	DH605	Z58
Amino acids and derivatives	Cycloleucine	1.02	1.16
	L-Asparagine	7.13	4.78
	L-Threonine	3.14	1.52
	L-Valine	1.37	1.41
	L-Serine	4.97	1.90
	Glutathione reduced form	2.71	3.73
	L-Ornithine	1.13	3.95
	Pipecolic acid	1.53	1.30
	L-Aspartic Acid	-3.29	-2.75
Organic acids	γ -Aminobutyric acid (GABA)	2.18	2.86
	L-Homoserine	3.16	1.33
	4-Guanidinobutyric acid	2.32	1.33
	Citric Acid	2.61	1.08
	Methylenesuccinic acid	-1.48	-1.90
	Shikimic acid	-2.08	-1.36
Phenolic acids	2-Methoxy-4-ethenylphenol	1.99	1.39
	Protocatechuic acid-4-O-glucoside	2.57	1.13
	1-O-Feruloyl-3-O-p-Coumaroylglycerol	2.21	1.92
	Demethyl coniferin	1.74	1.82
	1,3-O-Diferuloylglycerol	3.67	1.65
	5-Hydroxymethylfurfural	5.00	2.41
	6-O-Caffeoyl-D-glucose	2.72	3.93
Nucleotides and derivatives	Isopentenyladenine-7-N-glucoside	1.43	1.56
	2-Deoxyribose-1-phosphate	3.62	2.90
	Xanthine	4.13	2.12
	Guanine	2.24	1.18
	Cytidine 5'-monophosphate (Cytidylic acid)	12.01	1.30
Sugars	Turanose	2.04	1.51
	Nicotinate D-ribonucleoside	2.69	2.10
	D-Glucosamine	16.78	3.35
	Raffinose	1.08	2.17
	Gluconic acid	-2.89	-3.48
	D-Saccharic acid	-1.31	-1.59
Alkaloids	N-Caffeoylputrescine	2.89	1.18
	N-p-Coumaroyl-N'-feruloylputrescine	4.90	3.63
	p-Coumaroylputrescine	1.07	1.71
	Putrescine	21.69	3.53
	Diethanolamine	14.05	2.33
	N-Acetylputrescine	2.58	3.80
	6-Deoxyfagomine	1.04	1.32
	Piperidine	1.01	1.31
	Indole	1.29	1.47
Lipids	1-Methoxy-indole-3-acetamide	1.07	1.28
	13-KODE; (9Z,11E)-13-Oxooctadeca-9,11-dienoic acid	-1.01	-1.16
	1- α -Linolenoyl-glycerol-3-O-glucoside	-1.04	-1.09
	13S-Hydroxy-9Z,11E,15Z-octadecatrienoic acid	-1.02	-2.96

log2FC(T/C)

-3
-2
-1
1
2
3

Arginine metabolism under K⁺ deficiency

Remarkably, the top 30 pathways of different accumulated metabolites included “ABC transporters”, “biosynthesis of amino acids”, “glycine, serine, and threonine metabolism”, “carbon metabolism”, “2-Oxocarboxylic acid metabolism”, and “Aminoacyl-tRNA biosynthesis” (Fig. 4). Based on the above metabolome results, metabolites in arginine metabolism and the related metabolites were of interest due

to their accumulation under K⁺ deficiency treatment (Fig. 5). The related arginine metabolites, such as ornithine, agmatine, N-Acetyl-putrescine, 4-Acetamidobutyric acid and γ-Aminobutyric acid were also accumulated both in DH605 and Z58 (Fig. 5). Also, putrescine derivatives were accumulated as N-Caffeoyl putrescine, N-*p*-Coumaroyl-N'-feruloyl putrescine, *p*-Coumaroyl putrescine and N-Acetyl putrescine (Table 2). Additionally, the expression of arginine decarboxylase genes (*ADC*, *LOC100193626* and *LOC103638134*), mainly responsible for putrescine synthesis, were both upregulated in maize under K⁺ deficiency condition, which was consistent with the accumulation of putrescine in maize shoots (Fig. 5; Table S3).

Discussion

Fertilizer is important for plant growth and development and the related studies have been focused on different levels besides morphology, transcriptome, and metabolism (Ding et al. 2021; Hafsi et al. 2014; Ma et al. 2020; Mo et al. 2019; Pettigrew 2008). In the study, the K⁺ deficiency showed a significant decrease in maize aboveground biomass production in field and hydroponic experiments. The transcriptome and metabolism changes under long-term K⁺ deficiency treatment was further analyzed in the hydroponic experiments. Most DEGs were classified as associated with regulatory processes, transport, primary and secondary metabolism. DAMs induced by K⁺ deficiency was mostly involved in primary and secondary metabolism, such as amino acids and derivatives, sugars and sugar alcohols, organic acids, phenolic acids, alkaloids and nucleotides and derivatives. The results indicated that genes related to signal transduction, including MAKP signaling pathway and plant hormone signaling transduction, may play an important role as feedback to K⁺ limitation from shoot to the root.

Developmental responses to K⁺ deficiency

The K⁺ deficiency showed a significant decrease in silage maize production and the application of K⁺ fertilizer can increase total silage maize biomass, which indicated that K⁺ directly affects maize biomass yield (Perry et al. 1972). As for maize, most studies have focused on K⁺ in maize growth and their effects on grain yield rather than the whole plant biomass (Amanullah et al. 2016; Ul-Allah et al. 2020). Due to K⁺ deficiency, the maize plant height and stem diameter reduced, thus significantly limiting the plant growth, manifested as a decrease in fresh plant weight (Fig.2; Table S1). The physiological response under K⁺ deficiency was consistent with previous studies (Kanai et al. 2007; Ma et al. 2020). K⁺ plays important role in photosynthesis in the process of photosynthetic by converting radiant energy into chemical energy through the production of ATP and severe K⁺ deficiency can reduce photosynthesis (Kanai et al. 2011; Tester and Blatt 1989). Less efficient absorption of K⁺ in root dramatically reduces its content in shoots (accounts only 16%-20% of the control), which reduces photosynthesis and inhibits plant growth (Figs. 2 and 3)(Battie-Laclau et al. 2014; Ma et al. 2020). Moreover, the leaves and stems act as the sink of K⁺ and carbon assimilation in plant growth, and the retardation of plant growth is a feedback signal to K⁺ deficiency (Kanai et al. 2007). Similar responses to K⁺ deficiency was observed in tomato and sugarcane,

as well as in wood plants *Eucalyptus grandis* (Hartt 1969; Kanai et al. 2007; Ployet et al. 2019). Therefore, it is important to applying potassium fertilizer to improve the maize biomass production in agriculture, as well as to improving the plant K⁺ utilization efficiency in breeding.

Transcription responses to K⁺ deficiency

Several transcriptome profiles have been studied on plant responses to K⁺ deficiency (Ma et al. 2020; Ma et al. 2012; Ployet et al. 2019; Ruan et al. 2015; Yang et al. 2021; Zhao et al. 2018). The study analyzed the transcription changes of shoots in maize under K⁺ deficiency condition and a total of 1638 DEGs were identified in two varieties. Many of the previously reported transcriptional K⁺ stress responses, such as K⁺ transporters and ABC transporters, were tracked in DEGs of maize shoots. K⁺ transporters play crucial roles in translocation and cell growth in various plant species (Wang and Wu 2013). There are mainly three families in K⁺ transporters: the K⁺ uptake permeases (KT/HAK/KUP), the K⁺ transporter (Trk/HKT) family, and the cation proton antiporters (Gierth and Maser 2007). Consistent with the previous reports, the expression levels of *HAK1* and *HAK5*, belonging to high-affinity K⁺ transporters, were both up-regulated (Qin et al. 2019). The *AKT2* was also upregulated in the shoots of DH605 and Z58. The up-regulation of K⁺ transporters can increase K⁺ mobilization and promote the K⁺ uptake in root as well (Qin et al. 2019). A long-distance K⁺ transport would be important to redistribution and feedback the hungry status of potassium (Pilot et al. 2003). Additionally, the expression of several ABC transporters was also significantly induced in shoots, indicating that ABC transporters might be important for K⁺ and its related metabolism transport in plants under low K⁺ concentrations (Xie et al. 2020). Since yellow or brown edges and tips of leaves is a typical symptom of K⁺ deficiency, DEGs involved in aging and leaf senescence were also detected (Table S4). In addition to the above-mentioned genes, DEGs also includes those involved in biological process such as amino acid transport, amino sugar catabolic process, and response to nitrogen compound. The shortage of K⁺ supply limits plant leaf growth, probably due to sugar starvation in sink tissues such as stem and leaves (Hafsi et al. 2014).

Transcription factors have been identified to be indispensable in biotic and abiotic stresses (Ma et al. 2012; Zhang et al. 2017). The number of differentially expressed TFs for both varieties is 38 under K⁺ limitation condition, which included members from MYB, WRKY, bZIP, and PLATZ families (Table S5). Part of the TFs were predicted with MAPK signaling pathway and plant hormone signal transduction (Table S5). MAPK signaling pathway has been reported to be associated with senescence and apoptosis, which could explain the leaves with yellow or brown edges and tips under K⁺ deficiency condition (Sun et al. 2015). Genes involved in plant hormone signal transduction would affect their content and distribution to regulate plant growth (Ma et al. 2020). The results showed that brassinosteroid related genes, *TCH4* (*LOC100283318* and *LOC100284736*) was downregulated in both varieties, which played important roles in cell elongation (Takahashi et al. 2005). Besides, gene related with zeatin biosynthesis, like *type-B ARR* genes (*LOC107522107* and *LOC100280246*) were upregulated (Argyros et al. 2008). Therefore, the regulated TFs may play an important role in how maize shoots respond to K⁺ deficiency.

Metabolic responses to K⁺ deficiency

The responding metabolites of K⁺ stress comprised a broad range of metabolite classes, such as “ABC transporters”, “biosynthesis of amino acids”, “glycine, serine, and threonine metabolism”, “carbon metabolism”, “2-Oxocarboxylic acid metabolism”, “Aminoacyl-tRNA biosynthesis”, and so on. Of the metabolites, sugars, such as raffinose, turanose, and D-glucosamine, were accumulated, which were also detected in P deficiency and cold stress (Cook et al. 2004; Ding et al. 2021). Free amino acids (such as Asn, Thr, Val, Ser, Orn) increased in both varieties, while part of other amino acids (such as Arg, His, Leu, Pro) was only accumulated in either DH605 or Z58. K⁺ can activate enzymes and play important roles in protein synthesis (Hafsi et al. 2014). The content of amino acids increased during K deficiency condition, while the protein content reduced in cotton (Wang et al. 2012). Accumulation of free amino acids was also observed under P deficiency in plants (Ding et al. 2021; Hernandez et al. 2007; Mo et al. 2019; Pant et al. 2015). The results indicated that the deficiency of macronutrients in plants would suppress their carbon metabolism and affect the accumulation of amino acids. In addition, the amino acid transport related genes were responded to K⁺ deficiency, accompanied by an upregulation of genes involved in xenobiotic transmembrane transporter activity and xenobiotic transmembrane transport ATPase activity (Table S4).

Among the metabolites, 4-amino-butyric acid (GABA), putrescine and putrescine derivative were significantly accumulated under K⁺ deficiency (Fig. 5). Additionally, arginine decarboxylase gene, the key gene responsible for putrescine synthesis, was upregulated under K limited condition (Fig. 5). Putrescine accumulation is one of the metabolic marker in response to K⁺ deficiency in plants, which could reduce fast-activating vacuolar (FV) channel activity under stress (Bruggemann et al. 2002; Richards and Coleman 1952). Thus, the increased putrescine concentration and FV channel activity would maintain the cytosol K⁺ concentration (Walker et al. 1996). Putrescine has also been shown to be documented to be correlated with other stresses such as salinity and cold, as well as plant growth (Guo et al. 2019; Kou et al. 2018).

Conclusions

This study represents a multi-level study of maize shoots responses to K⁺ deficiency. The results suggest that the variation in transcription supports the physiological and developmental acclimation and adaptation to K⁺ deficiency. Since potassium play important roles in maize biomass yield, it is dispensable to improve the plant K⁺ utilization efficiency. The identification of differential transcriptomes and metabolomes under K⁺ deficiency and further research will be a foundation for future work in improving K⁺ utilization efficiency in breeding program.

Abbreviations

DEG: differently expressed gene

DAM: differential accumulated metabolite

VIP: variable importance in project

RPKM: transcript per million fragments mapped

GO: Gene Ontology

PCA: Principal component analysis

TF: Transcription factor

GABA: γ -amino-butyric acid

ADC: arginine decarboxylase genes

KEGG: Kyoto Encyclopedia of Genes and Genomes

Declarations

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Author contributions

SJ, SX, and XW conducted experimental design and manuscript writing. XW, WY, FD, WT, GY, YG, and CJ conducted in experiments and data collection. SJ, SX, XW, and WY analyzed the data. All authors approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

Biomass yield of silage maize as influenced by year and treatment (field experiment). K0 represents plots applying no potassium fertilizer; K1 represents plots applying potassium fertilizer.

Effect of K⁺ deficiency on maize growth. A and B showed phenotypes of maize DH605 and Z58 under K⁺ treatment for 12 days, respectively. C, D, and E showed the variations of average stem diameter, plant height, and plant fresh weight under K⁺ deficiency condition for 12 days, respectively. Two maize varieties, DH605 and Z58, were analyzed to K⁺ deficiency, respectively. CK and LK represent K⁺ sufficiency (4 mM) and K⁺ deficiency (0.1 mM), respectively. Scale bars in Figure 2A and Figure 2B represent 10 cm. The experiments were repeated three times. * and ** on histograms mean the significant difference at the P ≤ 0.05 and P ≤ 0.01 level, respectively. Bars mean SD.

Figure 3

Shoot potassium content (A), leaf net photosynthetic rate (B) and differential expression genes (C) of maize DH605 and Z58 under K⁺ deficiency treatment. CK and LK represent K⁺ sufficiency (4 mM) and K⁺ deficiency (0.1 mM). The experiments were repeated three times. * and ** on histograms mean the significant difference at the $P \leq 0.05$ and $P \leq 0.01$ level, respectively. Bars mean SD.

Figure 4

KEGG pathway analysis of DEGs under K⁺ deficiency treatment in DH605 and Z58. (A) KEGG pathway enrichment and top-level pathways of DEGs under K⁺ deficiency treatment in DH605. (B) KEGG pathway enrichment and top-level pathways of DEGs under K⁺ deficiency treatment in Z58. (C) The top-level pathways of DEGs under K⁺ deficiency treatment in DH605 and Z58 ($P \leq 0.05$).

Figure 5

KEGG analysis of DAMs and DEGs under K⁺ deficiency treatment ($P \leq 0.05$).

Figure 6

The metabolites that varied significantly in DH605 and Z58 after exposure to K⁺ deficiency. The first and second box of each metabolite indicate the varied metabolites of DH605 and Z58 after exposure to K⁺ deficiency, respectively. The first and second triangle of each gene indicate the varied genes of DH605 and Z58 after exposure to K⁺ deficiency, respectively.

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