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Research article

Impact of potassium deficiency on cotton growth, development and potential microRNA-mediated mechanism

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ABSTRACT

The goal of this study was to investigate the impact of potassium deficiency on cotton seedling growth and development at the individual, physiological, biochemical, and molecular levels. Potassium is an important plant nutrient; our results show that potassium deficiency significantly affected cotton seedling growth and development, evidenced by reduced plant height, and total areas of the leaves and roots as well as further reduced both fresh and dry biomass of the entire plants. Potassium deficiency also significantly inhibited root and leaf respiration and leaf photosynthesis. Compared with the controls, potassium deficiency significantly inhibited root elongation and total root surface areas that further inhibited cotton seedlings to uptake nutrients from the medium. Potassium deficiency induced aberrant expression of both microRNAs (miRNAs) and their protein-coding targets. These miRNAs regulate plant root development as well as response to abiotic stresses. Potassium deficiency altered the expression of miRNAs that regulate the expression of protein-coding genes controlling root development and response to potassium deficiency. miRNAs regulate root development and further control plant development in cotton seedlings under potassium deficiency. In summary, potassium deficiency significantly affected the cotton seedling photosynthesis and respiration that resulted in inhibition of cotton seedling growth and development potentially due to the miRNA-mediated mechanism.

1. Introduction

Cotton (*Gossypium hirsutum* L.) is a major fiber crop and is estimated to account for approximately 25% of the world's fiber usage (Johnson et al., 2018). Based on the U.S. Department of Agriculture's (USDA's) world cotton projections for 2018/2019, cotton production is expected to fall 3.6% due to low yields and decreasing agricultural land (Johnson et al., 2018). The demand for cotton is expected to steadily increase, and with low cotton yield, consumption will exceed production. In order to maximize cotton yield, the conditions in which it is grown is crucial. Cotton plants are often naturally subjected to potassium (K) deficiency resulting in developmental hindrances and further yield loss. Fortunately, plants have developed certain mechanisms to tolerate this stress through post-transcriptional regulation. Potassium is a macronutrient that is essential for many physiological and biochemical processes pertaining to plant growth, development, and nutrient uptake. Research shows the symplastic potassium concentrations are consistently between 80 and 200 mM (Ragel et al., 2019). Potassium controls many biological and metabolic processes that influence agricultural output, including water regulation, photosynthesis, and acting as an enzyme activator (Pettigrew, 2008). A previous research shows that a plant activated potassium uptake through K^+ transporters and channels when it was unable to obtain proper amounts of potassium (Hafsi et al., 2014). The plant also undergoes various signaling cascades similar to biotic stress responses. However, the exact change and molecular mechanisms are unclear. Understanding the morphological effects and molecular mechanisms of cotton undergoing potassium deficiency can ultimately be used to optimize crop production.

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Understanding cotton's molecular response to potassium deficiency by analyzing the expression of microRNAs (miRNAs) and their targeted genes can potentially aid in increasing crop yield.

miRNAs are an extensive class of small non-protein-coding RNAs with 21–24 nt in length, which play an important role in the post-transcriptional regulation of protein synthesis (Zhang et al., 2007). miRNAs can silence gene expression by splicing the messenger RNA (mRNA) or inhibiting mRNA translation in which the miRNA has complementary base-pairing with (Mallory and Vaucheret, 2004; Zhang and Unver, 2018). miRNAs are involved in many biological and metabolic processes, including plant development (Li and Zhang, 2016), hormone signaling (Liang et al., 2013; Mallory et al., 2005), responses to biotic (Prasad et al., 2019) and abiotic stressors (Song et al., 2019; Zhang, 2015); there are also several reports on the impact of fertilizers on miRNA expression in crops (Chien et al., 2017; Lu et al., 2014; Patel et al., 2017). miRNAs are also reported in cotton (Hu et al., 2020; Pan et al., 2019), in which the majority of studies are focused on cotton fiber development and response to different biotic (Hu et al., 2020; Pan et al., 2019) and abiotic stress, including drought and salinity (Ding et al., 2017; Gao et al., 2016; Xie et al., 2015). However, no any report on the impact of nutrients on miRNAs in cotton. In this study, we systematically investigated the impact of potassium deficiency on cotton at different levels, including individual, physiological, biochemical and molecular levels. We also studied the potential miRNA-mediated mechanism during these progresses. Observing the expressions of these miRNAs and their targeted genes in cotton plants with potassium deficiency can begin to reveal the elements involved in the mechanisms the plant undergoes to combat stress.

2. Materials and methods

2.1. Seed sterilization and germination

Healthy mature seeds of cotton (*Gossypium hirsutum* l.) cv Baimian No 1 were used in this experiment. Seeds were sterilized by soaking in 10% hydrogen peroxide for 20 min followed by washing with sterilized water for five times to remove the resides of hydrogen peroxide. The seeds were then rolled in a germination paper (20 seeds per roll) about 1 cm apart with the micropyle (tip) pointing down. Germination occurred in the plastic culture boxes (10 rolls per box) containing the ¼ strength of the modified Hoagland medium that contained the following components: NaCl (1 mM), $Ca(NO_3)_2$ (1.25 mM), KCl (2.5 mM), $NH_4H_2PO_4$ (0.25 mM), $MgSO_4$ (0.5 mM), EDTA FeNa (0.05 mM), H_3BO_3 (0.02 mM), $ZnSO_4$ (0.001 mM), $MnSO_4$ (0.001 mM), $CuSO_4$ (0.2 μM), $(NH_4)_6Mo_7O_{24}$ (0.005 μM). Punctured plastic covering was placed over the germination paper and containers to allow airflow and prevent water evaporation and was then placed in a dark room at 28 °C for 4 days.

After 4 days of germination, a total of 50 cotton seedlings were collected and transferred to a liquid culture system with ½ strength of modified Hoagland medium containing the following components: NaCl (2 mM), $Ca(NO_3)_2$ (2.5 mM), $NH_4H_2PO_4$ (0.5 mM), $MgSO_4$ (1 mM), EDTA FeNa (0.1 mM), H_3BO_3 (0.02 mM), $ZnSO_4$ (0.001 mM), $MnSO_4$ (0.001 mM), $CuSO_4$ (0.2 μM), $(NH_4)_6Mo_7O_{24}$ (0.005 μM). To determine the effect of potassium, two potassium concentrations were used in this study. The low potassium (deficiency) solution contained KCl (0.02 mM) and a normal potassium (control) solution contained KCl (2.5 mM). Both treatment and control groups of seedlings were cultured at 14/10-h light/dark cycle. The culture temperature was 30 ± 2 °C for light stage and 25 ± 2 °C for dark stage.

2.2. Effect of potassium deficiency on cotton biomass

After 4 and 8 days of treatment, six plants were collected from the potassium deficiency treatment and the control (the normal potassium concentration). Both fresh and dried weight were measured for each plant for both roots and up-ground parts.

2.3. Effect of potassium deficiency on root and leaf morphology and development in cotton

After 4 and 8 days of treatment, the leaves and roots were separated and scanned using the Epson Perfection V800 Photo scanner (Epsin America lnc, USA). Then, WinRHIZO 2007 (Regent Instruments Inc, Canada) was employed to analyze the area and volume of leaves, stems and roots. The height of plants and root length were also measured; the number of lateral roots were manually counted.

2.4. Effect of potassium deficiency on root vigor

Triphenyltetrazolium chloride (TTC) method was employed to measure root vigor. Dehydrogenase activity is an indicator of root vigor, which reduces TTC to triphenyltetrazolium formazan (TTF) that changes the color of the roots. Five centimeters of roots, including both main and lateral roots were removed from each plant from the bottom of the stem down along the main root; then, the remains were cut into pieces about 1 cm in length. After weighting, the root samples were soaked in a 15 mL tube with 0.1 M PBS (pH = 7.4) with 0.6% TTC. After the samples were treated at dark for 24 h, root samples were removed from the PBS buffer and washed using diH_2O for three times. Final, 5 mL 95% ethanol was used to extract the TTF from the roots at 85 °C water bath for 10 min. The light absorbance was measured at wave length of 485 nm. The root vigor was calculated using the following formula: root vigor = OD of 485 nm ÷ fresh weight (gram) of the samples.

2.5. Effect of potassium deficiency on cotton seedling respiration and photosynthesis

A polarographic O_2 electrode-Chlorolab 2 (Hansatech Instruments Ltd, England) was employed to monitor O_2 concentrations in the $CaSO_4$ solution with root or leaf samples. The respiration was monitored for 10 min and the respiration rate was calculated. The effect of potassium treatment on respiration was investigated in both leaf and root samples.

The kinetic and photosynthetic parameters of photosystem II (PSII) were estimated by means of the pulse-amplitude-modulation (PAM) measurement of chlorophyll fluorescence using the Dual PAM-100 Chlorophyll fluorometer (Heinz Walz GmbH, Germany). By measuring the rapid light curves (RLC) of the second fully opened leaves, the various chlorophyll-fluorescence parameters, namely ETRmax, alpha, I_K and MQY were derived by fitting a theoretical RLC. The ETR_{max} represents the relative maximum electron transport rate through the PSII complex, which is correlated with the overall leaf photosynthetic performance. Alpha is the initial slope of RLC, which reflects the quantum efficiency of the photosynthetic electron transport and relates to maximum yield of photosynthesis. Ik is the minimum photosynthesis-saturating irradiance and MQY (Fv/Fm × ETR factor/2) is the Maximum Quantum Yield representing the maximum PSII efficiency in the dark-adapted samples.

2.6. RNA extraction and gene expression analysis

Total RNAs were extracted from the roots of cotton plants at 4 and 8 days after the potassium deficiency treatment and the controls using the mirVana miRNA Isolation Kit (Ambion, Austin, TX) according to the protocol provided in the kit. Extracted RNAs were measured and the quality was evaluated using a Nanodrop ND-1000 (Nanodrop Technologies, Lnc, USA), and the samples were stored at −80 °C until the gene expression analysis.

A total of 1000 ng RNAs were first reversely transcribed into cDNA by using gene specific primers for miRNAs (Supplemental Table S1) and a pol(T) primer for all protein-coding genes, including the reference

genes. Then, the gene specific forward and reverse primers (Supplemental Table S1) were used for quantitative real time PCR (qRT-PCR).

In this experiment, 20 miRNAs and their targets were selected to investigate the environmental stress response due to potassium deficiency. These 20 miRNAs (the corresponded target) were miR160 (ARF10), miR164 (NAC1), miR165 (HD ZiP), miR166 (HD ZiP), miR167, miR 169 (NFYA), miR171, miR390 (ARF3), miR393 (TIR1), miR 396 (bHLH74), miR 847, miR857 (LACCASE7), miR156 (SPL3), miR162, miR 172 (AP2), miR 319, miR 395, miR778, miR 399, and miR 827. According to previous studies, these miRNAs were associated with plant root development and/or with homeostasis of nitrogen or phosphorus in other species as well as responses to other abiotic stresses (Song et al., 2019; Zhang, 2015). For qRT-PCR data analysis, *actin* and *elongation factor 1a* (*ef1a*) were used as reference genes to normalize the expression values of miRNAs and their targets. The fold change and gene expression for each miRNA and its target gene were calculated using the ΔΔCT method.

2.7. Biostatistical analysis of physical characteristics and gene expression changes

After taking the measurements of the physical characteristics of the cotton plants as well as evaluating the fold changes of the miRNA and their target gene expression levels, the results were analyzed using the statistical software SPSS version 22. A one-way ANOVA test for significant was performed on all four or five biological replicates between the treatment and the controls with each measurement (i.e. root weight, leaf weight). All treatments were tested with a 95% confidence interval.

3. Results

3.1. Potassium deficiency affected the growth and development of cotton seedlings

Potassium deficiency significantly affected cotton seedling growth and development. After 3 days of treatment, the plants with potassium deficiency treatment started to see the difference, including the changes in leaf color, plant shape and size as well as root development. It clearly saw that after 4 days of treatment, control plants grew quickly but the plants with potassium deficiency treatment grew very slowly.

Potassium deficiency significantly reduced the fresh plant biomass, including roots, stems and leaves (Table 1). After 4 days of treatment, the weight of control plants already reached to 1956, 1103, and 1086 mg/plant for leaves, stems, and roots, respectively. However, the plant with potassium deficiency treatment, the fresh weight was only 510, 392, and 319 mg/plant for leaves, stems, and roots, respectively. After 8 days of treatment, control plants grew much bigger, the fresh weight was 3783, 1729, and 2134 mg/plant for leaves, stems, and roots, respectively. However, for the potassium deficiency treatment, the plants almost no more grew, the fresh weight was only 551, 398, and 293 mg/plant for leaves, stems, and roots, respectively. The total weight for control plants was 4145 and 7646 mg/plant for 4 and 8 days of culture, respectively, with 84.5% increased. However, the total weight for plants treated with potassium deficiency was 1221 and 1242 mg/plant for 4 and 8 days of culture, respectively, with only 1.7% increased.

Potassium concentrations also significantly impacted the dry biomass when comparing the controls with the potassium deficiency treatments. As the plants continued to be treated for four and eight days in the hydroponic system, the difference between the controls and the treatments increased. The dry weights of four-day's leaves, stems, and roots were not significantly different comparing the controls with the potassium deficiency treatments. After eight days of treatment, the dry biomasses of leaves, stems, roots, upper ground parts, and total dry biomasses were all significantly inhibited with a p-value < 0.001 by the potassium deficiency compared to the controls (Table 1). The average leaf dry biomass per seedling for the eight days in the low potassium was 113 mg, which was significantly lower than the 2236 mg of the control. The stem biomass for the four days in low potassium was 27 mg and not significantly lower than the control. However, the eight days stem in low potassium had an average biomass of 45 mg, which had an significant decrease compared to the control. Similarly, average root biomass for the eight days in the low potassium was 19 mg, which was significantly lower than the control. Overall, cotton seedling dry biomass obviously decreased in the low potassium treatment as time progressed.

3.2. Potassium deficiency affected root development in cotton seedlings

Potassium deficiency significantly affected root development (Table 2, Fig. 1). Potassium deficiency not only affected the total biomass of root, but also affected root length, size, and the number of branches. No matter after 4 or 8 days of potassium treatment, potassium deficiency significantly inhibited root development, including the total root length, total root surface area, and total root volume. Under potassium deficiency, cotton roots also grew much smaller than the control. This effect was enhanced as increasing treatment time; after 8 days of treatment, although the cotton seedlings were still survival under potassium deficiency, they nearly stopped growing. However, the root size and total root volume were almost doubled under the control condition.

3.3. Potassium deficiency affected plant height and leaf development in cotton seedlings

Under the normal culture condition, after the cotton seedlings were transferred to the liquid culture, cotton leaves quickly grew and expended; in a short 4 days of period, the total leaf area was expended form 36.82 cm^2/plant to 99.54 cm^2/plant with 170% increase; the 8 days-old leaves were almost three time bigger than the 4 days-old leaves (Table 3). Although plant leaves also grew under potassium deficiency treatment, the leaves grew very slowly, during 4 days of culture period, the leaves only expended from 25.15 cm^2/plant to 35.97 cm^2/plant with only 43% increase in leaf area, it is more slowly than the control plants.

Potassium treatment also affected cotton plant height and branching (Table 3). After 4 days of treatment, the plant height was 10.66 cm for the treatment with potassium deficiency; however, the control plants

Table 1
Effect of K deficiency on cotton plant growth and biomass (mg/plant)[a],*.

DAT[b](d)	Treatments	FLW	FSW	FRW	DLW	DSW	DRW
4	Control	1956 ± 284a	1103 ± 124a	1086 ± 124a	76 ± 15a	33 ± 8a	22 ± 5a
	K deficiency	510 ± 74b	393 ± 39b	319 ± 77b	64 ± 14a	27 ± 3a	19 ± 4a
8	Control	3783 ± 476a	1729 ± 201a	2134 ± 139a	236 ± 26a	119 ± 12a	61 ± 11a
	K deficiency	550 ± 108b	398 ± 99b	293 ± 51b	113 ± 19b	45 ± 3b	19 ± 6b

*: Different lower-case letters at same DAT indicate significant difference between the control and K deficiency at $P < 0.05$ level.
[a] Fresh leaf weight (FLW); Fresh stem weight (FSW); Fresh root weight (FRW); Dry leaf weight (DLW); Dry stem weight (DSW); Dry root weight (DRW).
[b] DAT: day after treatment (DAT).

Table 2
Effect of K deficiency on cotton root development *,[a]

DAT[b] (d)	Treatments	TRL (cm/plant)	TRSA (cm^2/plant)	TRV (cm^3/plant)	ARD (mm)
4	Control	737.64 ± 58.07a	90.44 ± 5.10a	0.89 ± 0.09a	0.39 ± 0.03a
	K deficiency	254.42 ± 19.84b	32.26 ± 3.52b	0.33 ± 0.05b	0.40 ± 0.01a
8	Control	1675.71 ± 448.31a	171.94 ± 48.73a	1.41 ± 0.42a	0.33 ± 0.03b
	K deficiency	250.70 ± 10.95b	30.79 ± 1.68b	0.31 ± 0.02b	0.40 ± 0.01a

*: Different lower-case letters at same DAT indicate significant difference between the control and K deficiency at $P < 0.01$ level.
[a] Total root length (TRL), Total root surface area (TRSA), Total root volume (TRV), Average root diameter (ARD).
[b] DAT: day after treatment (DAT).

grew up to 12.34 cm with 1.68 cm higher than the potassium deficiency. After 8 days of treatment, this difference became 2.81 cm in which the control grew up to 15.39 cm but the treatment with only 12.58 cm. After 4 day of treatment, cotton seedling. also grew branching; potassium treatment also affected the number and length of branch. At 4 days of treatment, the total branch length (total stem length minus plant height) was 5.64 cm for the control, however it was only 3.35 cm for the potassium deficiency treatment. As increasing the treatment time, this difference became bigger. At 8 days of treatment, the total branch length was 12.03 and 4.62 cm for the control and potassium deficiency treatment, respectively.

3.4. Potassium deficiency affected root vigor as well as root and leaf respiration in cotton

Root plays unique role for the plant, which not only uptakes water and nutrients from the soil, but also responses to different environmental stresses as well as anchors the plant on the ground. The health of root can be monitored by many different ways, including the root vigor and respiration. For a short time period of potassium treatment (4 days), the root vigor did not show difference between potassium deficiency treatment and the controls. However, as increasing the treatment times and plant growth, although both treatment and control, the root vigor was increased, the control plants had much higher root vigor than the plants under potassium deficiency treatment (Table 4).

Table 3
Effect of K deficiency on plant height and leaves development in cotton seedlings *.

DAT (d)	Treatments	Total leaf surface area (cm^2/plant)	Total stem length (cm/plant)	Plant Height (cm)
4	Control	36.82 ± 9.44a	17.98 ± 2.02a	12.34 ± 1.05a
	K deficiency	25.15 ± 4.59b	14.01 ± 0.93b	10.66 ± 0.54b
8	Control	99.54 ± 9.04a	27.42 ± 1.68a	15.39 ± 2.37a
	K deficiency	35.97 ± 6.16b	17.20 ± 1.71b	12.58 ± 0.13b

*: Different lower-case letters at same DAT indicate significant difference between the control and K deficiency at $P < 0.05$ level.

Respiration is also an indicator for plant vigor. At the 4 days of treatment, the root respiration was higher for potassium deficiency treatment than the controls ($p < 0.05$). However, after 4 days of treatment, the root respiration was increased in the control plants, but it was decreased in the plants with potassium deficiency treatment. When reaching the 8 days of treatment, the root respiration was significantly higher for the control plants than that for potassium deficiency treatment. However, for the leaf respiration, it shows a different pattern. At 4 days of treatment, the control plants had higher respiration rate than that for potassium deficiency treatment; after that, the

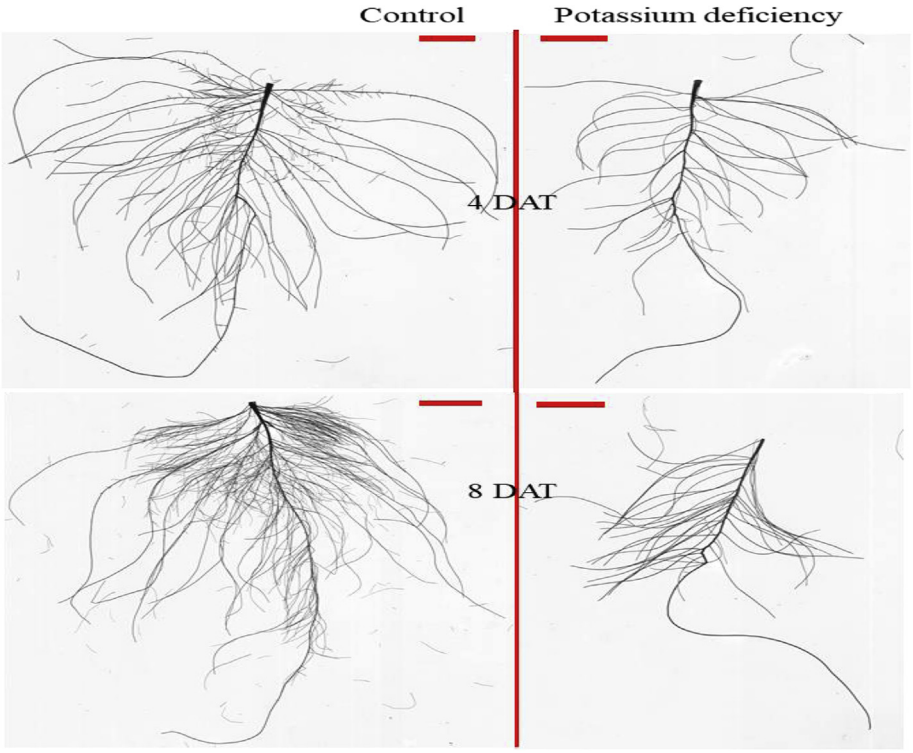

Fig. 1. Effect of K deficiency on cotton root development. DAT: days after treatment (DAT).

Table 4
Effect of K deficiency on leaf or root respiration reflected by oxygen consuming rate and root vigor at different day after treatment (DAT) under different K levels *.

DAT (d)	Treatments	Root vigor (OD/g FW)	Root	Leaf
			O_2 consuming rate (nM O_2/min/g FW)	
4	Control	0.42 ± 0.07a	35.55 ± 9.86b	498.86 ± 158.69a
	K deficiency	0.55 ± 0.08a	130.24 ± 21.67a	167.51 ± 29.36b
8	Control	5.23 ± 0.32a	83.94 ± 3.52a	70.92 ± 4.43b
	K deficiency	1.26 ± 0.18b	58.55 ± 9.90b	147.75 ± 56.58a

*: Different lower-case letters at same DAT indicate significant difference between the control and K deficiency at $P < 0.05$ level.

respiration rate was decreased in the control leaves, but it was increased in the treatment plants. After 8 days, the respiration rate was significantly higher in the potassium deficiency treated-leaves that in the control leaves.

3.5. Potassium deficiency affected leaf photosynthesis

Photosynthesis is a unique trait for plants. The chlorophyll fluorescence is a widely used tool to indicate photosynthetic performance (Jebali et al., 2019; Kalaji et al., 2004). The rate of photosynthesis plus other factors control plant biomass and further plant yield. In this study, we found that potassium deficiency treatment significantly affected cotton leaf photosynthesis being reflected by the PSII activities. In details, at the 4 and 8 days of treatment, K deficiency significantly reduced the ETR_{max} and I_K (Table 5), elucidating that K deficiency severely inhibited cotton plant growth (Tables 1 and 2).

3.6. Potassium deficiency altered the expression of miRNAs and their targets

All tested miRNAs and their targets were expressed at both potassium deficiency treatments and the controls at both time points (Fig. 2). At 4 days of potassium treatment, the majority of tested miRNAs were up-regulated by potassium deficiency treatment. However, as treatment going, the expression of most tested miRNAs was inhibited in potassium deficiency treatment except miR156, miR167 and miR393 (Fig. 2a). At 4 days of treatment, the most induced miRNAs were miR171 followed by miR167. At 8 days of treatment, miR393 was induced with 2.13-fold increase whereas miR171 was highly inhibited by more than 80% followed by miR162, miR857, and miR778 with more than 50% inhibition, respectively.

Not only was miRNA expression affected by potassium levels and treatment time, the expression of miRNA targeted genes was also greatly affected (Fig. 2b). At 4 days of potassium treatment, potassium deficiency induced the expression of the majority of tested protein-coding genes. Among the 10 tested miRNA targets, *tir1* gene was shown the most fold changes with 12.98-fold increases compared with the normal potassium treatment, followed by *bHLH74* and SPL3 transcription factor genes with 3.23- and 2.36-fold changes, respectively. Among the 10 test miRNA targets, NAC1 transcription factor gene shows decrease in expression and NFYA didn't show change in expression at 4 days of potassium treatment. As the potassium treatment going, the expression of target genes was changed. After 8 days of treatment, the expression of target genes was decreased for the majority of genes; among them, NFYA transcription factor show the most decrease in potassium deficiency treatment with about 4 folds in decrease compared with the controls. The expression of ARF10 and NAC1 transcription factor genes was only about half in potassium deficiency treatment compared with the controls. However, the expression of HD-ZiP and ARF3 transcription factor genes was slightly increased.

3.7. The positive and negative relationship between miRNAs and their targets under potassium deficiency treatment

miRNAs do not directly regulate plant growth and development as well as response to environmental stress. miRNAs regulate almost all biological and metabolic processes in plants through targeting protein-coding genes for translation repression or/and degradation of mRNAs (Mallory and Vaucheret, 2004; Zhang, 2015; Zhang et al., 2007). Thus, generally speaking, the expression of miRNAs should be reversed as the expression of their targets. In another words, it means the expression of a miRNA target should be inhibited if the expression of a miRNA is increased at a specific growth condition, such as abiotic stress. In this study, we also observed that the expression of at least four pairs of miRNAs and their targets are opposite direction (Fig. 3a). After 8 days of potassium treatment, the expression of miR165, miR166, and miR390 was inhibited; however, the expression of their target HD-Zip and ARF3 was increased. In contrast, the expression of miR393 was increased but the expression of miR393-targeted *tir1* gene was decreased. This suggests that miRNAs function through regulating their target genes.

However, some miRNAs and their targets did not always show the reverse correlation between their expressions. This is because that gene regulation is a complicated mechanism, except a miRNA targets a protein-coding gene, many other factors, such as DNA methylation and feedback regulation also affect gene expression. Although the complicated regulation mechanism, we can see a clear negative relationship between the expression of miRNAs and their targets (Fig. 3b). The expression change between miRNAs and their targets show a negatively liner relationship with a liner equation: $y = -0.5593x + 0.7898$ ($R^2 = 0.3179$). This suggests that as increase the expression of certain miRNAs, the expression of their targets is decreased and demonstrated the regulation pattern between miRNAs and their targets.

4. Discussion

Potassium is an important major nutrient for plant growth and development. During plant growth and development, cotton plant needs to uptake lots of nutrients, including potassium, from the soil or other medium. When not enough potassium resources available, a plant

Table 5
Effect of K deficiency on chlorophyll fluorescence of PSII at 8 day of treatment *.

Treatment	MQY (electrons/photons)	Alpha (electrons/photons)	ETR_{max} (μmol electrons/m^2/s)	I_K (μmol photons/m^2/s)
Control	0.32 ± 0.01a	0.39 ± 0.02a	41.27 ± 5.35a	106.3 ± 12.20a
K deficiency	0.30 ± 0.01b	0.40 ± 0.03a	27.03 ± 1.63b	67.15 ± 1.91b

*: Different lower-case letters at same column indicate significant difference at $P < 0.05$ level.

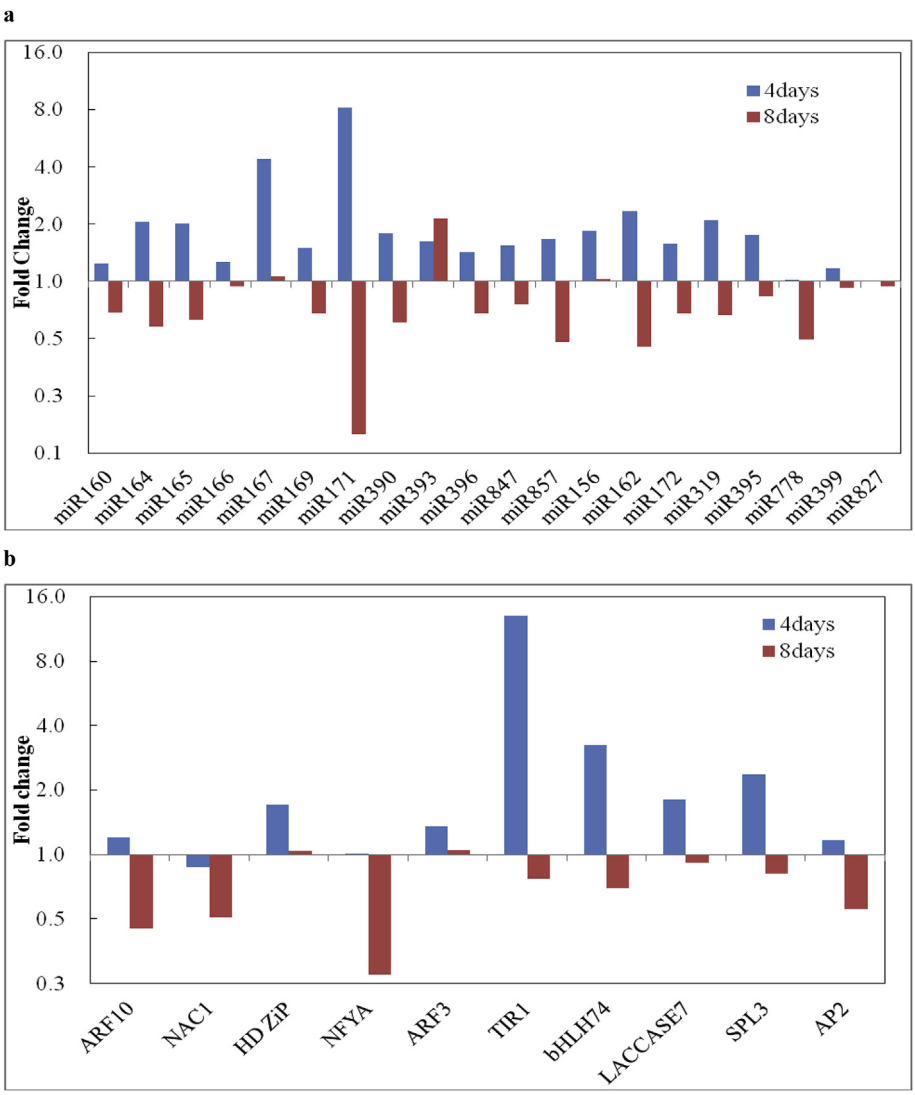

Fig. 2. Potassium deficiency induced aberrant expression of (a) miRNAs and (b) their targets.

employs different mechanisms to uptake more from the soil or water, that includes but not limits to: 1) enhancing root development to uptake more potassium from the soil; 2) regulating enzyme activities to increase potassium usage efficiency; 3) reutilizing potassium from old leaves to maintain plant growth; and 4) slowing plant growth. During potassium deficiency, cotton seedlings attempted to enhance the root development in order to update more potassium from the culture medium and to increase the root activities. However, this still cannot meet the need of cotton plant growth and development and finally resulted in aberrant growth and development of cotton seedling plants. These phenomena were also observed in other plant species under potassium deficiency treatment (Hafsi et al., 2017; Janpen et al., 2019; Li et al., 2019; Zeng et al., 2018). Potassium deficiency also decreased the biosynthesis of chlorophyll *a* and *b* and further affected photosynthesis which in turn reduced plant growth and plant biomass.

Root respiration provides the driving force for root growth and development as well as the absorption, maintenance, and transport of ion in a plant cell and between plant cells (Jia et al., 2013). At the plant level, potassium deficiency enhanced root respiration (Singh and Blanke, 2000); our study shows that potassium deficiency also increased respiration in both cotton seedling roots and leaves at 4 and 8 days of treatment (Table 4). This suggests a shift in plant metabolism required for K uptake or compensation of the increased stress. The respiration rate of plant correlates with both substrate supply and demand for respiratory energy. Soluble sugars deficit lowered the respiration rate in maize root tips (Saglio and Pradet, 1980), which might also explain the dramatic decrease of cotton root respiration from 4 days to 8 days of potassium deficiency (Table 4). High root respiration usually means high root activity showed by TTC reduction because TTC replaces O_2 as final acceptor of H^+/e^{-1} contributed by dehydrogenases catalyzing the oxidation of organic compounds. However, when facing potassium deficiency, the positive relationship of root vitality and respiration became weak (Table 4). There are several reasons to explain this phenomenon. First, potassium is an activator of more than 60 enzymes (Wang et al., 2013), and its deficiency might inhibit some dehydrogenase activity. Second, potassium deficiency lowered cytoplasmic and apoplastic pH (Li and Zhang, 2016; Walker et al., 1998), which might decrease dehydrogenase activity, and further TTC activities. Third, root activity and respiration rate were influenced by different root chemistry and anatomy, and even with different directions, which were observed in this study. Increased cell respiration and decreased leaf photosynthesis impacted plant metabolic pathways and enzyme activities, and further affected nutrient uptake from soil and transported between different tissues and organs. All of these inhibited plant growth and development and reduced plant biomass.

Potassium deficiency-induced plant physiological and morphological changes are caused by molecular change. A little bit of environmental changes, such as abiotic and biotic stresses, can induce the

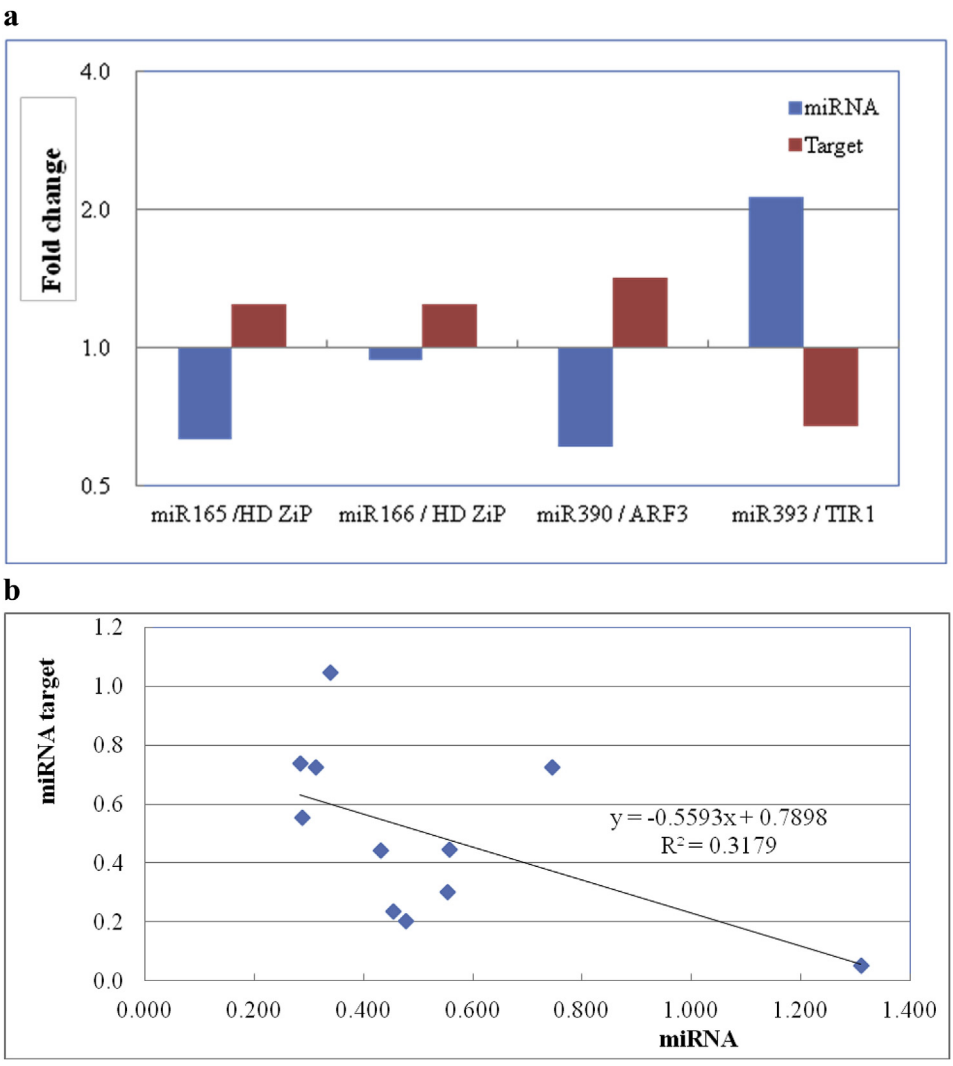

Fig. 3. Negative relationship between the expression of miRNAs and their targets. (a) The expression of 4 representative miRNAs and their targets at 8 days of potassium deficiency treatment. (b) Liner relationship between miRNAs and their targets. X axis represents the expression change of miRNAs from 4 days of treatment to 8 days of treatment; Y axis represents the expression change of miRNA targets from 4 days of treatment to 8 days of treatment.

aberrant expression of many genes in several plant species, including the small regulatory miRNAs (Chang, et al. 2020; Priya et al., 2019; Zhang, 2015). Although protein-coding genes have been frequently studied during potassium deficiency treatment, few studies on miRNA have been reported under nutrient deficiency stress. In a recent study, Zeng et al. (2019) identified a bench of miRNAs and their targets in two barley cultivars during potassium deficiency (Zeng et al., 2019); these miRNAs includes miR156 and miR393 that were also studied in this research.

miRNAs play versatile functions in both plant and animal systems (Mallory and Vaucheret, 2004; Zhang, 2015; Zhang et al., 2007). In plants, miRNAs control almost all biological and metabolic progresses, including tissue/organ development, timing, and response to biotic and abiotic environmental stresses. In this study, we investigated the impact of potassium deficiency on the expression of miRNAs and their targets. These miRNAs are associated with plant development, particularly on root development (Fig. 4). For example, miR160, miR164, and miR390 regulate lateral root development. At the early stage (4 days) of potassium deficiency treatment, the expression of these miRNAs were increased and the plants show enhanced root growth and differentiation for up taking more nutrients from the medium. However, as treatment going, the expression of these miRNAs were decreased at 8 days of treatment, and resulted in decreased biomass of roots. Thus, although root growth and development was enhanced at early stage of potassium deficiency, finally the root growth and development was inhibited by potassium deficiency. This further resulted in less nutrient uptake and inhibited growth of the entire plants.

CRediT authorship contribution statement

Julia Elise Fontana: Formal analysis, Writing - original draft, Writing - review & editing. **Guo Wang:** Formal analysis, Writing - original draft, Writing - review & editing. **Runrun Sun:** Formal analysis, Writing - review & editing. **Huiyun Xue:** Formal analysis, Writing - review & editing. **Qian Li:** Formal analysis, Writing - review & editing. **Jia Liu:** Formal analysis, Writing - review & editing. **Kyle E. Davis:** Formal analysis, Writing - review & editing. **Thomas Elliott Thornburg:** Formal analysis, Writing - review & editing. **Baohong Zhang:** Formal analysis, Writing - review & editing. **Zhiyong Zhang:** Formal analysis, Writing - review & editing. **Xiaoping Pan:** Formal analysis, Writing - review & editing.

Declaration of competing interest

The authors declare that there is no any competing interest.

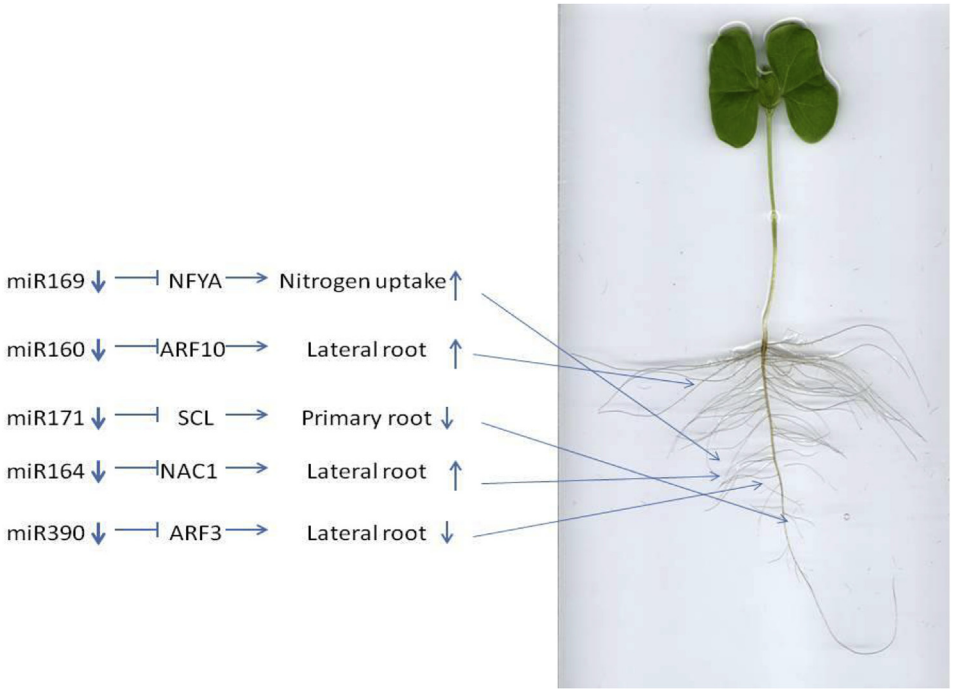

Fig. 4. miRNAs regulate root development and nutrient uptake through controlling the expression of protein-coding genes.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2020.05.006.

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