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Potassium deficiency limits reproductive success by altering carbohydrate and protein balances in cotton (*Gossypium hirsutum* L.)

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Highlights

- K deficiency limited the sucrose and amino acids translocation to the pistils.
- K deficiency decreased markedly carbohydrate and protein metabolism in pistils.
- K deficiency decreased pollen tube growth and fertilization efficiency.
- Low reproductive success was related to the changes in pistils biochemistry under K deficiency.

Abstract: Reproductive success in higher plants requires a lot of energy and substance provided by carbohydrate and protein metabolism，and potassium (K) plays an important role in carbohydrate and protein metabolism. However, it is unclear whether K deficiency limits reproductive success by disturbing carbohydrate and protein metabolism. The objectives of this study were to explore the effects of K deficiency on carbohydrate and protein metabolism in subtending leaves, phloem and pistils, and their relationship with reproductive success. A cotton cultivar DP0912 was grown in K-deficient (0 mM K^+) and K-sufficient (6 mM K^+) nutrient solution in growth chambers. Results showed that *Pn* of the subtending leaves was decreased under K deficiency, but sucrose, starch and free amino acid contents were markedly increased in the K-deficient leaves, because K deficiency limited the translocation of sucrose and amino acid in phloem. As a result, sucrose and free amino acid contents were reduced by 47.3% and 51.8% in the K-deficient pistils than K-sufficient pistils, which led to further decreases in starch and protein accumulation in the K-deficient pistils. Glucose content was also reduced by 53.1% in the K-deficient pistils than K-sufficient pistils, due to the decreased acid and alkaline invertase activities, since sucrose synthase activity was not affected. Lastly, soluble carbohydrate and ATP contents were lower in the K-deficient pistils than K-sufficient pistils, similarly to the changes of pollen tube growth rate and seed set efficiency. It was concluded that the lower carbohydrate and ATP contents in the K-deficient pistils could not meet the energy requirements of pollen tube growth and seed set. Moreover, protein imbalance also limited pollen tube growth. Those changes

resulted in lower seed set efficiency to limit reproductive success.

Keywords: Cotton (*Gossypium hirsutum* L.); K deficiency; Carbohydrate balance; Protein balance; Reproductive success

1. Introduction

Flowering is very important for final yield (Boyer, 1982) and quality (Ruan et al., 2001) for crops with reproductive structures. Herrero and Johnson (1981) reported that drought stress during pollination could greatly reduce kernel set of maize (*Zea mays* L.) by affecting female and male floral development. The number of kernels per unit land area in wheat (*Triticum aestivum* L.) was significantly decreased when higher than optimum temperature occurred right before flowering (Fischer, 1985). Similarly, the number of seeds per locule in cotton (*Gossypium hirsutum* L.) were decreased under high night temperature stress during the flowering stage (Echer et al., 2014). Abdullah et al. (2001) noticed that reduced seed set in the panicle as a result of decreased pollen viability under salt stress resulted in low yield per plant in rice (*Oryza sativa* L.). Thus, environmental stresses can affect reproductive characteristics during flowering to restrict the formation of yield components.

Flowering is a critical event for the reproductive development in cotton, and is very sensitive to abiotic stresses (Snider et al., 2011a). Poor environmental or nutritional conditions during flower development are likely the main reasons for the difference between actual and potential yield in cotton (Snider et al., 2009). Potassium (K) deficiency as a common abiotic stress could strongly decrease cotton yield (Oosterhuis et al., 2013) by reducing boll number (Li et al., 2012) and boll weight (Hu et al., 2016a). Past studies have also indicated that K deficiency markedly reduced yield by decreasing boll (capsule wall, seed and lint) biomass (Hu et al., 2015; Reddy and Zhao, 2005). Additionally, K deficiency might significantly decrease cotton yield by inhibiting reproductive success, since K^+ contributes to pollen germination and tube growth (Fan et al., 2001) due to its pivotal role in turgor pressure regulation (Rehman and Yun, 2006). Furthermore, during floral development, carbohydrate and protein concentrations play vital roles in reproductive success (Chang et al., 2009). In vivo, the pollen grain first utilizes the stored carbohydrates and proteins to germinate (an autotrophic phase), then absorbs external carbohydrates and amino acids originating in the style to grow (a heterotrophic phase) (Gonzalez et al., 1996; Chang et al., 2009). The majority of carbohydrates supplied to reproductive organs come from the subtending leaf of cotton, which has been reported to supply more than 87% of the total carbohydrate requirement of the boll (Ashley, 1972). Furthermore, Wang et al. (2012) reported that sucrose and amino acid metabolism would be altered by K deficiency in cotton leaves. Under K deficiency, the activity of enzymes involved in carbohydrates and amino acid synthesis were decreased (Hu et al., 2015; Hu et al., 2016c), but total nonstructural carbohydrates and free amino

acid concentrations were significantly increased in leaves (Hu et al. 2017). Zhao et al. (2001) speculated the inconsistency was probably due to a restricted export of assimilation products by inhibition of some of the steps involved in phloem loading (Marschner et al., 1996). Consequently, the restricted export of assimilation products may limit carbohydrate and protein supplies to sinks (Hu et al. 2017), including flowers.

A previous study reported that insufficient supply of assimilates is one of the main reasons for the abscission of flowers (Stewart, 1986) and similar results were reported by Snider et al. (2009) who observed that the low total soluble carbohydrate content in pistils resulted in the reduction of fertilization rate and the increase of abortion rate of cotton bolls. In addition, low soluble sugar and starch contents in rice spikelets would significantly increase spikelet sterility (Fu et al., 2010). Furthermore, there was clear evidence that protein content and amino acid composition in ovaries were related to ovary setting (Ghiasi et al., 1987), and in vivo pollen tube growth utilized the protein stored in the transmitting tissue of the style (Gonzalez et al., 1996). Snider et al. (2011b) noticed that environmental stresses could alter carbohydrate balance and protein reserves in cotton pistils to affect reproductive success, but studies investigating the effects of K deficiency on reproductive success by altering carbohydrate and protein metabolism during anthesis are lacking.

Therefore, we hypothesized (1) that K deficiency would alter carbohydrate and protein balance in pistils simultaneously restrict sucrose and amino acid export from the leaf, and (2) that reproductive success would be decreased due to the aforementioned changes in pistil biochemistry under K deficiency. The objectives of this study were (1) to investigate the effects of K deficiency on pistil biochemistry and (2) to quantify the effects of changed carbohydrate and protein metabolism in the pistil on reproductive success under K deficiency.

2. Materials and methods

2.1. Experimental design

Experiments were conducted in January and were repeated in July 2015 using a cotton cultivar DP0912 (Monsanto Company, St. Louis MO) at the Altheimer Laboratory, University of Arkansas. Seeds were planted in sand filled 2-L pots in growth chambers (Conviron PGW36, Conviron Inc., Winnipeg, Manitoba, Canada) under day/night temperatures of 30/25 °C, a photosynthetic flux density of 800-850 μmol m^{-2} s^{-1}, a relative humidity of 60% and a 12/12 h photoperiod. The Hoagland's nutrient solution contained 6 mM K^+, 14 mM NO_3^-, 2 mM NH_4^+, 4 mM Ca^{2+}, 2 mM PO_4^{3-}, 2 mM SO_4^{2-} , 2 mM Mg^{2+}, 1 mM Fe^{3+}, 46 μM H_3BO_3, 7.3 μM Cl^-, 3.7 μM Mn^{2+}, 0.77 μM Zn^{2+}, 0.32 μM Cu^{2+}, and 0.12 μM MoO_3. All pots were alternately watered with 1/4 strength K nutrient solution (1/4 strength K in Hoagland's solution using NH_4NO_3 in place of KNO_3) and distilled water until flowering. Two K treatments (each treatment had 29 pots) including (1) a sufficient K supply treatment (6 mM K^+)

as a control and (2) K deficiency treatment (0 mM K^+) were initiated at the beginning of the flowering stage.

2.2. Sampling and processing

White flowers and their subtending leaves at the first fruiting position of fruiting branchs at the 7–10th mainstem node above the cotyledons fruiting branches were subsequently used as experimental units, because plants from the K deficiency treatment showed severe K deficiency symptoms at that time and the bolls on the 7–10th fruiting branches could make an important contribution to yield (Oosterhuis, 2001). The subtending leaves and pistils were sampled at 11:00. Some leaves were used for phloem exudation measurements, and others were dried at 105 °C for 30 min and heated at 70 °C for 72 h to dry for measuring carbohydrate contents and leaf K content. For biochemical analysis and K concentration analysis, twelve pistils were immediately frozen in liquid nitrogen and stored at -80 °C. Four pistils were immediately used for ATP extraction. Six pistils were collected at 11:00 and 13:00, and stored in formalin acetic acid-alcohol (FAA) for subsequent microscopy, and the remaining flowers were tagged to note the date. According to the method of Donohue et al. (1992), the K content in leaf and pistil was assayed by the Soils Testing Laboratory, University of Arkansas, Fayetteville.

2.3. Photosynthesis measurements

Photosynthesis of the leaves subtending the flower was measured using a CI-340 hand-held photosynthesis system (CID Bio-Science, Inc., Camas, WA, USA) at 9:00-10:30. The clear-top chamber set was 30 °C temperature, 400 μmol^{-1} CO_2 concentration and 60% relative humidity. Before measuring the photosynthesis for each leaf, 5 min was needed to reach steady state after the leaf sample was enclosed in the chamber.

2.4. Carbohydrates, free amino acid and soluble protein concentrations

Carbohydrate contents of leaves and pistils were extracted and assayed according to Snider et al. (2011b) with slight modification. 40 mg of ground tissue was put into a tube before adding 1 mL of 80% (v/v) ethanol. The tube was heated at 80 °C three times, and the three supernatants were collected together and 80% ethanol was added to a total volume of 3 mL. Activated charcoal was added before centrifuging at 1164 g for 15 min to remove compounds that interfere with the determination of carbohydrate. For analysis, 20 μL of extract was added to a 96-well microtitration plate. Then the plate was heated at 50 °C to evaporate the ethanol before adding 20 μL distilled water into each well. The plate was continuously incubated three times with 100 μL glucose assay reagent [glucose (HK) assay kit; Sigma] at 30 °C for 15 min, with 10 μL phosphoglucose isomerase (0.25 units, Sigma) at 30 °C for 15 min, and with 10 μL invertase (83 units, Sigma) at 30 °C for 60 min, respectively. After each incubation step, the absorbance was determined at the wavelength of 340 nm with a microplate reader (Molecular Devices Corporation, Sunnyvale, CA) to determine the concentration of glucose, fructose, and sucrose.

The ethanol-insoluble residue was collected for starch extraction. After adding 0.5 mL of 1 M KOH, the samples were heated at 100 °C for 1 h. Then adjust pH to 6.5-7.5 using 0.2 M acetic acid before adding 100 μL a-amylase to each sample. The samples were incubated at 65 °C for 60 min. The pH of each sample was decreased less than 5 using 1M acetic acid before adding 0.25 mL amyloglucosidase. After incubating at 55 °C for 60 min, the samples were centrifuged at 10,000×g for 15 min. Then the supernatant was collected and deionized water was added to a final volume of 3 mL. 20 μL extract was added into a 96-well microtitration plate to determine glucose concentration according to the above method. The concentration was calculated according to Zhao et al. (2008).

Fresh leaf (0.3 g) or pistil (0.15 g) was used for the extraction of free amino acid and soluble protein according to Hu et al. (2016c). The free amino acid content was assayed according to the ninhydrin method (Yemm et al., 1955) with slight modification. 1.5 mL extraction was added into a tube before adding 0.5 mL of 3% (w/v) ninhydrin in 95% (v/v) ethanol. The samples were heated at 100 °C for 10 min before adding 5 mL of 95% (v/v) ethanol. The absorbance was detected at 570 nm and the free amino acid content was expressed as mg g^{-1} fresh weight (FW). The soluble protein content was determined by Bradford reagent (Bradford, 1976). 0.1 mL extraction was pipetted into a test tube. Then 5 mL coomassie brilliant blue G-250 solution was added to the test tubes before vortexing. The absorbance at 595 nm was measured. Bovine serum albumin was used to make a standard curve.

2.5. Adenosine triphosphate (ATP) measurement

Fresh pistils were homogenized with 5 mL of 50 mM Tris–HCl solution (pH 7.3), and then were transferred to the tubes. The tubes were heated at 100 °C for 10 min before centrifuging at 21,000×g for 10 min. The supernatant was collected for ATP quantification. The ATP bioluminescent assay kit (Sigma Chemical Company, ST. Louis, MO, USA) was used, which converted the chemical energy associated with ATP into light and luminescence was detected with a 20/20n Luminometer (Turner Bisosystems Inc., Sunnyvale, CA, USA) (Loka and Oosterhuis, 2016). ATP content was expressed as μg g^{-1} after luminescence of samples was compared with that of standards having known ATP concentrations.

2.6. Enzyme extraction and analysis

The extraction of pistil enzymes was according to Huber and Israel (1982). Sucrose synthase (SuSy, E.C. 2.4.1.13) activity was measured according to Chen et al. (2014). The reaction mixtures (650 μL) contained 2 mM UDP-glucose, 100 mM sucrose, 20 mM Pipes-NaOH (pH 7.5), 50 mM $MgCl_2$ and 200 μL extract. Reactions were started by incubating at 30 °C for 30 min and were terminated by heating at 100 °C for 10 min with 250 μL of 0.5 M Tricine-KOH. The content of fructose was measured spectrophotometrically.

Soluble acid and alkaline invertase (EC 3.2. 1.26) activities were measured by incubating 100 μL extract, 200 μL of 1 M sucrose and 2.2 mL of 200 mM acetic acid-NaOH (pH 5.0, for acid invertase), or 100 mM sodium acetate-acetic acid (pH 7.5, for alkaline invertase) at 30 °C for 30 min. The reaction was terminated by adding 1 mL 3,5-dinitro salicylic acid before boiling for 5 min. The absorbance was detected at 540 nm and used to calculate enzyme activity according to Chen et al. (2014).

2.7. Phloem export of sucrose and free amino acid

Phloem exudate was collected from the subtending leaves using the EDTA method (Wang et al., 2012). The cut ends of the petioles were put into 10 mL EDTA solution (20 mM, pH 6) for 15 min in the dark. The first EDTA solution was abandoned to avoid contamination by xylem exudates, and then the petioles were washed and transferred to 10 mL of 20 mM fresh EDTA solution. In order to avoid transpiration, the solution was immediately placed in the dark for 5 h. The final exudation solutions were used for determining the concentration of sucrose and free amino acid according to the above methods (Yemm et al., 1955; Snider et al., 2011b).

2.8. Pollen viability, pollen tube growth rate and seed set efficiency

pollen viability was assayed using the TTC (2,3,5-Triphenyltetrazolium chloride) staining method (Cao et al., 2012). Pollen was placed into a 1 % TTC solution for 30 min for microscopic examination. For pollen tube growth rate determination, pistils were collected at 11:00 and 13:00. Styles were excised using a scalpel and placed in a fixation solution of FAA. For softening styles, the styles were put in 10% anhydrous sodium sulfite and heated at 100 °C for 20 min, and then transferred into 10% $NaHCO_3$ solution prepared with 30% alcohol at room temperature. After 2 hours, the styles were rinsed with distilled water several times. Styles were stained with 0.025% aniline blue (Bradbury, 1929). Pollen tubes were observed under a UV microscope. The seeds with 5-6 mm fibers in the bolls of 5 days after anthesis were considered as normal developing seeds. Seed set efficiency was calculated as follows: (number of normal developing seeds per ovary) / (total number of ovules in each ovary) ×100.

2.9. Statistical analysis

No significant differences were observed between the first experiment and the repeated experiment, and the results were pooled. Statistical analysis was performed by SPSS statistic package Version 17.0 (Hu et al., 2015) using a t-test. Means were considered significantly different at $P \leq 0.05$. All figures were made using Origin 8.0 software.

3. Results

3.1. Leaf K concentration and subtending leaf physiology

Leaf K concentration of the subtending leaf was markedly decreased for the K-deficient plants by 42.2% compared to the control (Fig. 1A). Additionally, K deficiency resulted in a significant decrease in *Pn* (Fig. 2), since *Pn* of the subtending leaf for the K-deficient plants was only 19.7% of the control plants. Sucrose content increased substantially in the subtending leaf of K-deficient plants having 103.4% higher content of sucrose than that of control plants (Fig. 3A). A similar pattern was observed for starch content, which was immediately affected by K deficiency since starch content of the subtending leaf was significantly increased by 61.8 % for the K-deficient plants compared to the control (Fig. 3B). Free amino acid content in the subtending leaves of the K-deficient plants exhibited a marked increase relative to that of control plants (Fig. 3C).

3.2. Export of sucrose and free amino acid

Under K deficiency, the export of sucrose should be reduced considerably in phloem, and the actual level of export was lower in the K-deficient treatment than control treatment based on a unit leaf or a unit FW basis (Table 1). When the export capability of the leaf was evaluated using the ratio of exported sucrose content in phloem to sucrose content in the leaf (phloem:leaf ratio) per unit DW, sucrose export was acutely impaired by K deficiency. In contrast with the trend of free amino content in leaf, amino acid export in phloem was substantially reduced by K deficiency, since the amino acid export per hour per leaf or per hour per leaf fresh weight was decreased by 36.7% or 41.0% in the K-deficient treatment than control treatment. Based on phloem:leaf ratio, the free amino acid export in the K deficiency treatment was decreased by 85.4% compared to the control.

3.3. Pistil K concentration and reproductive competence

Pistil K concentration was 36.2% lower under K deficiency compared to the control (Fig. 1). Additionally, K deficiency did not cause a significant change in pollen viability shown as 87.6 % and 89.0% viable pollen in the K-deficient treatment and the control treatment, respectively (Table 2). A different trend was observed for pollen tube growth. Pollen tube growth rate of K-deficient pistils was decreased by 40.9% compared to the control (Table 2). The number of ovules per ovary of K-deficient plants was not significantly different than that of control plants ($P > 0.05$). However, seed set efficiency was adversely impacted by K deficiency, since it declined by 19.0% relative to the control (Table 2).

3.4. Pistil physiology

Carbohydrates contents of pistils were assayed and the results indicated that glucose, sucrose, starch and total soluble carbohydrate were negatively affected by K deficiency, decreasing by 53.1%, 47.3%, 49.7% and 28.9% relative to the control, respectively (Fig. 4 and Fig. 5A). However, there was no significant difference between the K deficiency treatment and control for pistil fructose content (Fig. 4). K deficiency also resulted in a significant

reduction in ATP content (Fig. 5B). ATP content of pistils exposed to K deficiency was approximately 53.2% lower than that of pistils supplied with optimum K quantity. Free amino acid and soluble protein contents of pistils were also decreased by 51.8% (Fig. 6A) and 45.1% (Fig. 6B), respectively, under K deficiency relative to the control. Sucrose synthase, acid invertase and alkaline invertase are important enzymes for sucrose degradation. Acid and alkaline invertase activities decreased substantially under K deficiency, by 49.9% and 24.9%, respectively, compared to the control (Fig. 7B and 7C). However, sucrose synthase activity of K-deficient pistils was not significantly different than that of pistils grown under optimum K supply (Fig. 7A).

A significant positive correlation between total soluble carbohydrate content and pollen tube growth rate as well as seed set efficiency (P <0.05) was identified (Table 3). Similarly, soluble protein and ATP content were positively correlated with pollen tube growth rate and seed set efficiency (P <0.05).

4. Discussion

Potassium deficiency significantly decreased K concentration in the subtending leaves (Fig. 1A), which could impair metabolic processes (Hu et al., 2015). We observed that *Pn* of the subtending leaves for the K-deficient plants was only 19.7% of the plants with sufficient K supply (Fig. 2), which was consistent with the results reported by Hu et al. (2016b). Previous studies have indicated that the effects of K deficiency on *Pn* are interrelated with stomatal or non-stomatal limitations, depending on the growth stage of cotton (Hu et al., 2016b) and the severity of K stress (Wang et al., 2012). Disruptions in metabolic functions such as photosynthesis and respiration, have been reported to result in changes in plant carbohydrate concentrations (Loka and Oosterhuis, 2016). Along with declines in *Pn*, both sucrose and starch contents should be significantly lower under K dificiency. However, the results from the present study did not support the theoretical prediction because sucrose and starch contents were higher in the K-deficient leaves (Fig. 3A and 3B). Zhao et al. (2001) speculated that this phenomenon was due to restricted sucrose export from source to sink under K deficiency. Nitrate reductase and glutamine synthetase activities have been reported to be decreased by K deficiency (Ruiz and Romero, 2002; Armengaud et al., 2009), leading to reduced N assimilation in *Arabidopsis* root (Armengaud et al., 2009) and marked decreases in free amino acid content in tea (*Camellia sinensis* L) leaves (Ruan er al. 1998). In contrast to that, in our study, free amino acid content was significantly increased in the K-deficient leaves compared to the control (Fig. 3C). Similar results of increased free amino acid concentration under low K stress were also reported in maize leaves (Hsiao et al., 1970). We also speculated that altered amino acid metabolism was related to restricted export rate in phloem.

Photoassimilate export has been observed to decline before decreases in photosynthesis are observed under K

deficiency (Gerardeaux et al., 2010). In the present study, the results indicated that sucrose export in phloem based on per leaf, per unit FW of leaf or the phloem:leaf ratio, was significantly reduced under K deficiency (Table 1), suggesting that K deficiency markedly restricted sucrose's phloem loading. Cakmak et al. (1994) reported comparable results in exepriments with bean (*Phaseolus vulgahs* L.), where phloem structure of source needles was severely disorganized and became necrotic under K deficiency. Amino acid transport rate followed a similar pattern declining by 36.7% and 41.0% under K deficiency relative to the control when evaluated on the basis of per leaf and per unit FW (Table 1). The reduction was even more severe when the amino acid transport rate was based on the ratio of phloem:leaf, indicating that K deficiency also markedly limited the transport of amino acids. Wang et al. (2012) reported that phloem export of amino acids is strongly correlated with sucrose loading and mass flow in the phloem, and a fixed ratio of amino acids to sucrose has been observed in the cytosol of phloem (Cakmak et al., 1994), leading us to conclude that reductions in sucrose export due to K deficiency led to restrictions in the transport of amino acids. Moreover, the restricted export of sucrose and amino acids in phloem might disturb carbohydrate and protein metabolism in sinks (Hu et al. 2017), and the pistil is one of the main sinks at anthesis.

The results presented in Figs. 4–7 indicated that disturbing carbohydrate metabolism and protein metabolism in the K-deficient pistils were obvious. For example, lower soluble carbohydrate content was measured in the K-deficient pistils (Fig. 5A) relative to the control, which was mainly due to lower sucrose and glucose contents in K-deficient pistils since no significant difference in fructose content was observed (Fig. 4). We speculate that the decreased glucose content, observed in our study was due to the reduced sucrose hydrolysis, since both acid and alkaline invertase activities were markedly lower in the K-deficiency pistils compared to the control, while sucrose synthase activity was not affected (Figs. 7A, 7B and 7C). Hu et al. (2015) reported that leaf acid invertase and sucrose synthase activities were substantially increased and decreased, respectively, under K deficient conditions, which was in contrast with our results probably due to the different tissue types, and leaves belong to the source and pistils belong to the sink. Pistil sucrose content, similar to glucose content, was significantly lower in the K-deficient pistils, which was mainly attributed to the decreases in sucrose transport rates from leaf to pistil. Consequently, sucrose-to-starch conversion was significantly decreased (Yang et al., 2004) in the K-deficient pistils, resulting in lower starch content in the K-deficient pistils compared to the control (Fig. 4). Pistil protein content depends on amino acids accumulation and their transport rate from the leaf to the pistil. Lower than optimum K supply has been reported to influence amino acid transport (Cakmak et al., 1994) and that appears to be the case in our study, where amino acid export rates of K deficiency treatment were markedly lower compared

to the control. Consequently, amino acid levels of K deficient pistils were significantly decreased leading to reduced protein synthesis and ultimately lower pistil protein content compared to the control (Figs. 6A and 6B). Furthermore, K deficiency significantly decreased pistil ATP content (Figs. 5B), which is in contast with the results obtained by Bednarz and Oosterhuis (1999). In their experiment, higher ATP content was observed in the K-deficienct leaves, leading them to speculate that ATP utilization might be restricted more than ATP formation in K-deficient leaves. However, in the K-deficient pistils, lower ATP content should result from lower ATP formation duo to lower carbohydrate content.

The seed set efficiency was 23.0% lower under K deficiency than control (Table 2). However, there were no significant differences in the number of ovules per ovary between K deficiency treatment and control treatment, suggesting that the lower seed set efficiency might result from low efficiency of pollination (Peet et al., 1998), which was related to a decline in the amount of pollen available to pollinate a receptive stigma (Snider et al., 2009), pollen viability (Kakani et al., 2005), the growth of pollen tubes toward ovules (Snider et al., 2011a) or the ovules themselves not being receptive (Saini et al., 1983). In the present study, pollen viabitity was not affected, but pollen tube growth rate was decreased by 40.9% under K deficiency relative to the control. Therefore, we suggested that the lower seed set efficiency under K deficiency could be explained by the declines in pollen tube growth in the style. Developing pollen grains were a major sink of carbohydrates (Min et al., 2013), and pollen tube growth in the style requires sufficient carbohydrate supply (Gonzalez et al., 1996). Additionally, Snider et al. (2011b) indicated that pollen tube growth rate is highly correlated with the concentrations of pistil sucrose, fructose, and soluble carbohydrates, while any stress limiting carbohydrate allocation to the pistil could decrease reproductive success (Tadege and Kuhlemeier, 1997). In the present study, soluble carbohydrate content in the K-deficient pistils declined concomitantly with pollen tube growth, and significant positive correlations were observed between soluble carbohydrate content and pollen tube growth rate as well as seed set efficiency, leading us to suggest that the energy requirements of pollen tube growth could not be met in the K-deficient pistils. In support of this observation, significant correlations were identified between ATP and pollen tube growth rate as well as seed set efficiency. In addition, previous studies also indicated that efficient utilization of protein reserves in the style is required for successful pollen tube growth (Herrero and Arbeloa, 1989; De Graaf et al., 2001). Taking into consideration lower protein content in the K-deficient pistils and the significant positive correlations between protein content and pollen tube growth as well as seed set efficiency (Table 3), the lower seed set efficiency in the K deficiency treatment was a direct result of the lower protein content of the pistils. Similar results have been reported by Carbonell-Bejerano et al. (2010) in experiment with *Arabidopsis*, where protein

level was obviously lower in the unfertilized pistils than the fertilized pistils.

5. Conclusion

In conclusion, K deficiency significantly affected carbohydrate and protein metabolism of cotton leaves subtending the flower. Despite lower *Pn* was observed in the K-deficient leaves than K-sufficient leaves, sucrose, starch and free amino acid contents were markedly increased, since K deficiency limited the sucrose and amino acids export from the K-deficient leaves. As a result, sucrose and amino acid levels of K-deficient pistils were markedly decreased compared to the control leading in turn to lower starch and protein accumulation. Pistil soluble carbohydrate content was also affected by K deficiency with glucose and sucrose levels being significantly lower compared to the control, and lower glucose was mainly due to the reductions in the activities of both acid and alkaline invertase. In addition to the decreased soluble carbohydrate content, pistil ATP level was reduced under lower than optimum K conditions. Consequently, the energy requirements of pollen tube growth and seed set were unable to be met under K deficiency. Moreover, low protein content in pistil could not meet the protein demand of successful pollen tube growth in vivo. Those results caused lower seed set efficiency limiting reproductive success.

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Figure legends

Fig. 1 Effects of K deficiency on (A) the subtending leaf and (B) pistil K concentrations. Columns followed by different letters are significantly different at *P*=0.05 level. All values are means of four replications ± standard error.

Fig. 2 Effect of K deficiency on net photosynthesis (*Pn*) of the subtending leaf. Columns followed by different letters are significantly different at *P*=0.05 level. All values are means of four replications ± standard error.

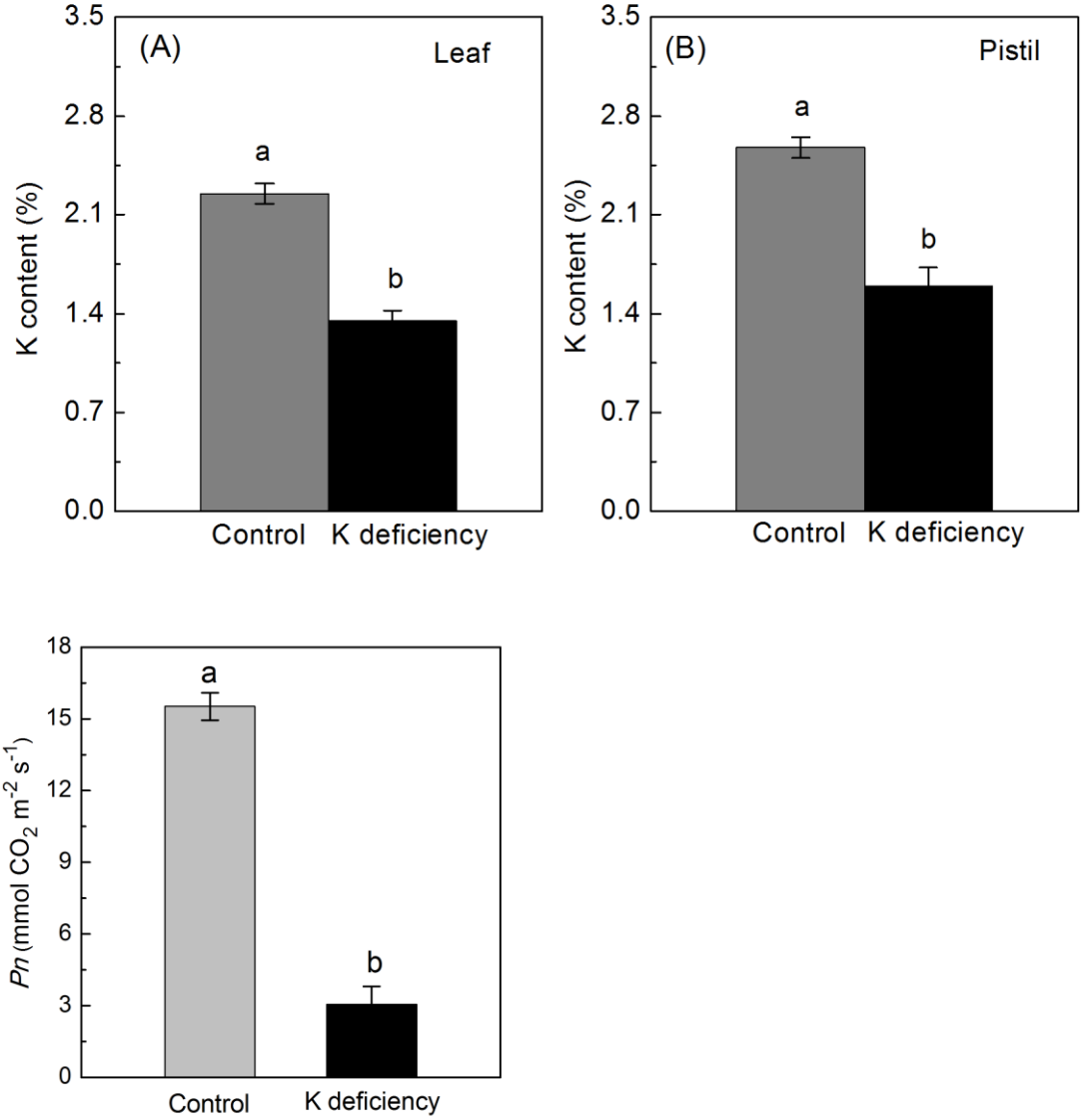
Fig. 3 Effects of K deficiency on (A) sucrose, (B) starch and (C) free amino acid contents of the subtending leaf. Columns followed by different letters are significantly different at *P*=0.05 level. All values are means of four replications ± standard error.

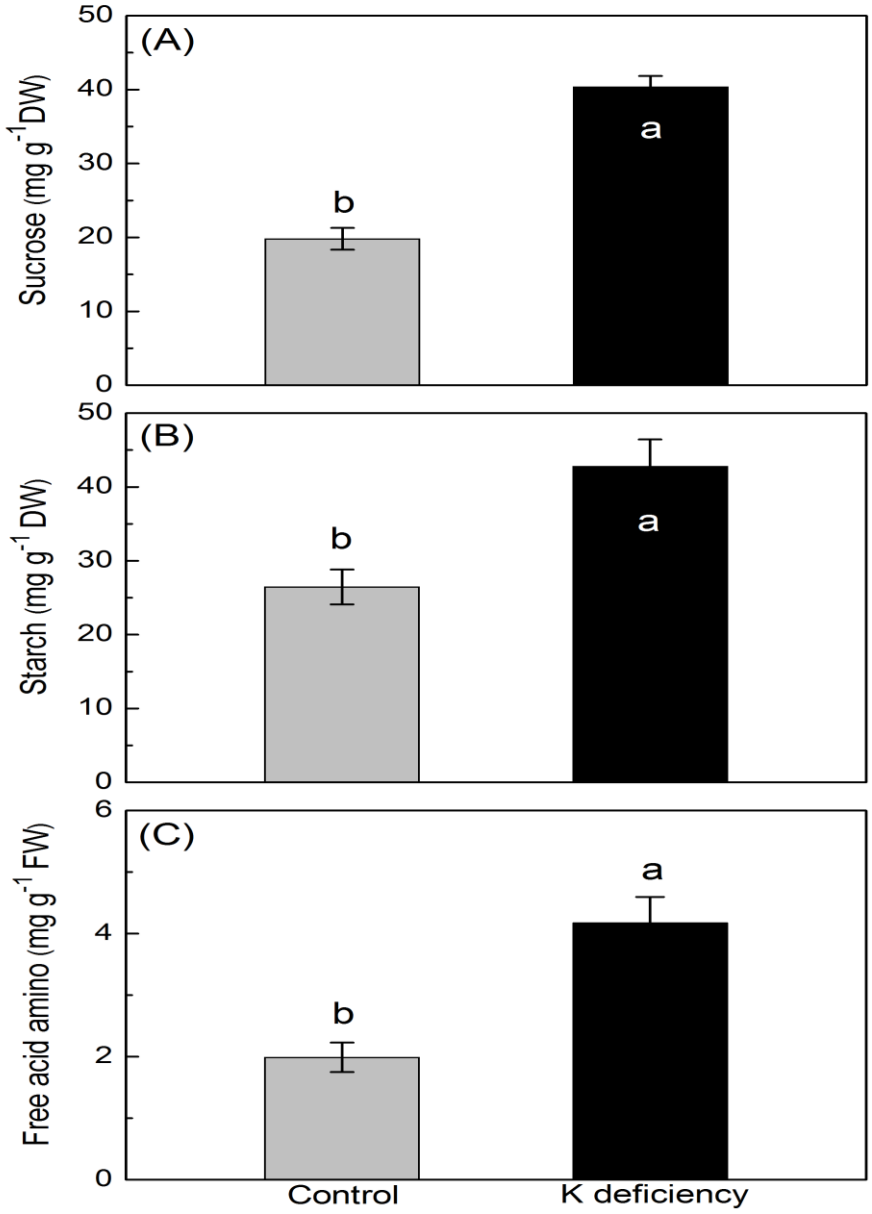
Fig. 4 Effects of K deficiency on glucose, fructose, sucrose and starch contents of the pistil. Columns followed by different letters are significantly different at *P*=0.05 level. All values are means of four replications ± standard error.

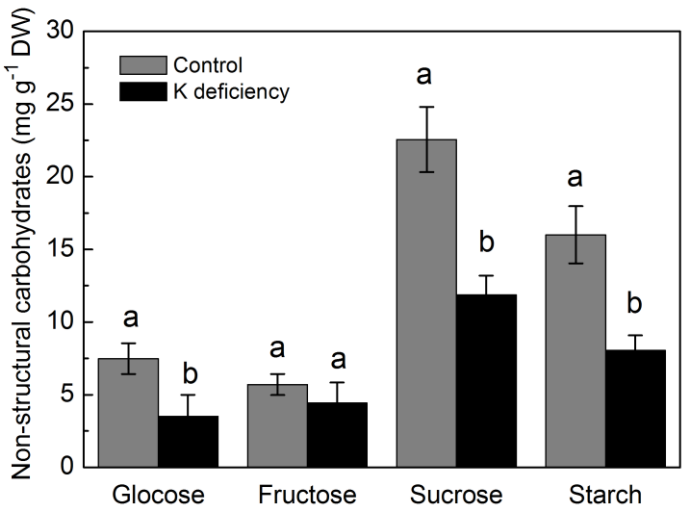
Fig. 5 Effects of K deficiency on (A) total soluble carbohydrate content (B) and adenosine triphosphate (ATP) content of the pistil. Columns followed by different letters are significantly different at *P*=0.05 level. All values are means of four replications ± standard error.

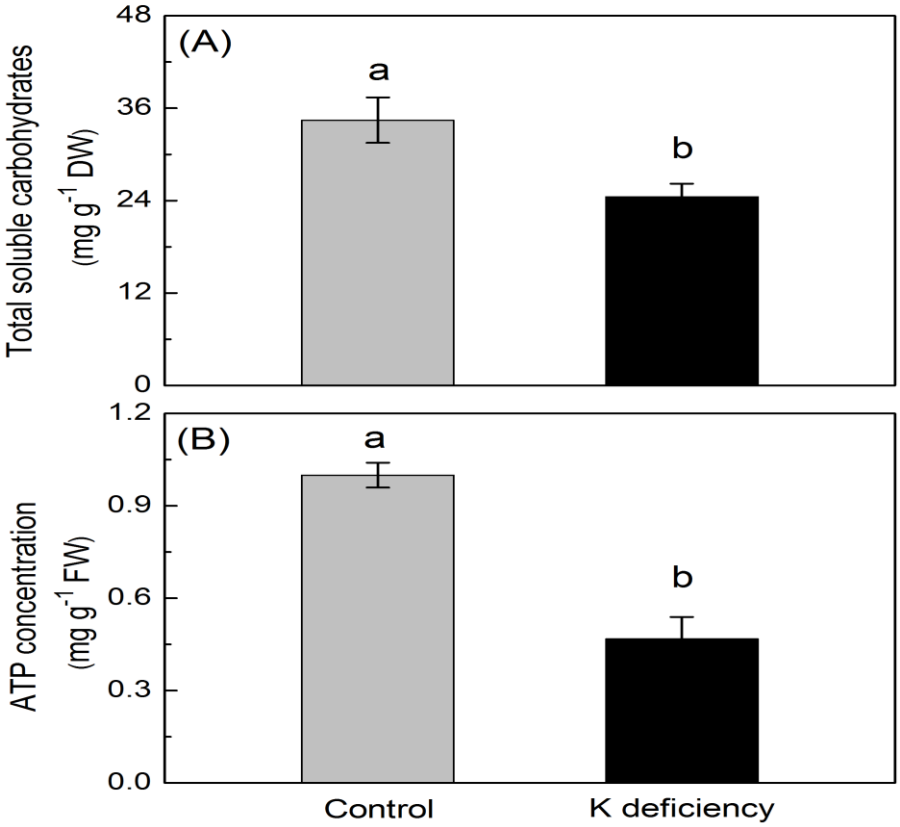
Fig. 6 Effects of K deficiency on (A) free amino content and (B) soluble protein content of the pistil. Columns followed by different letters are significantly different at *P*=0.05 level. All values are means of four replications ± standard error.

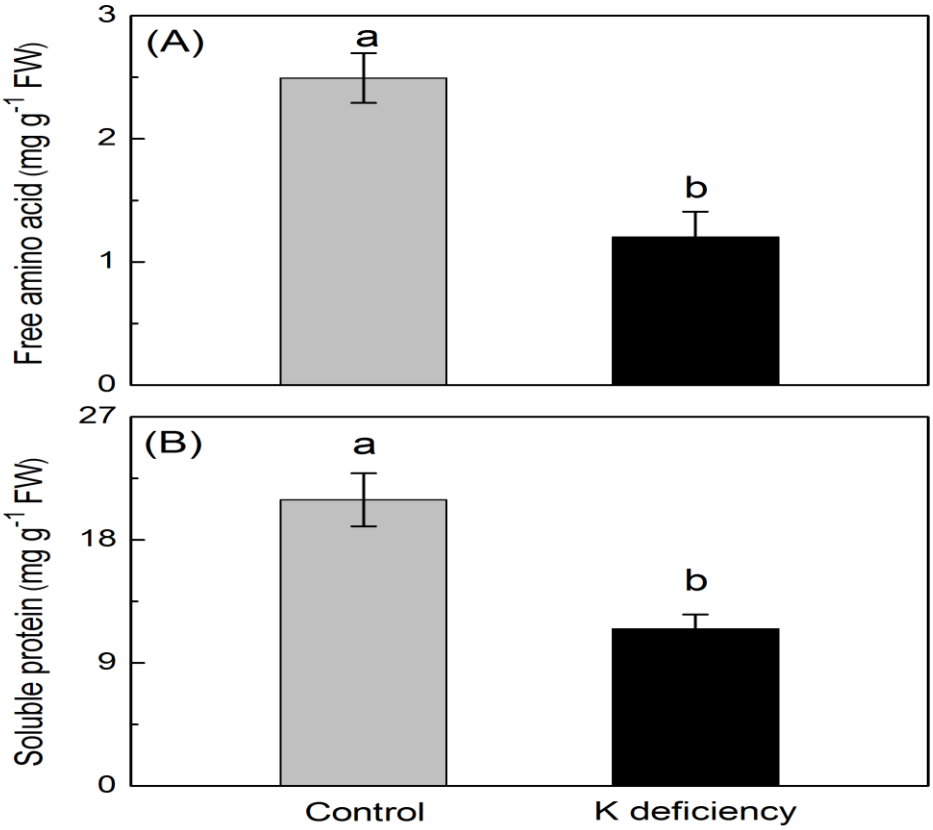
Fig. 7 Effects of K deficiency on (A) sucrose synthase activity, (B) acid invertase avtivity and (C) alkaline invertase activity of the pistil. Columns followed by different letters are significantly different at *P*=0.05 level. All values are means of four replications ± standard error.











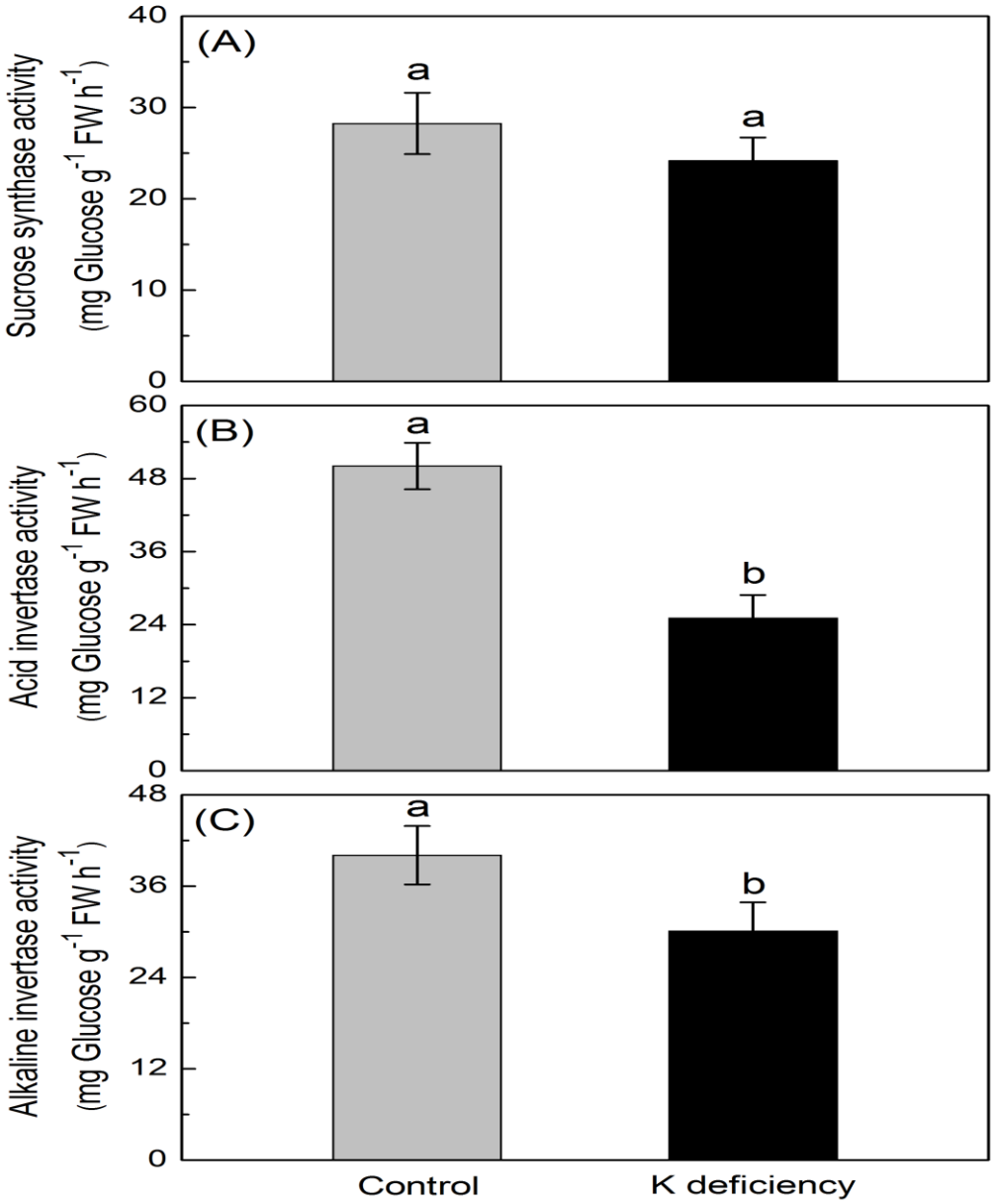


Table 1 Effect of K deficiency on phloem export of sucrose and free amino acid. Phloem exudates were collected from detached leaves using the EDTA-promoted technique. All values are the means of four replications ± standard error. ** indicates that the differences between two treatments are significant at 0.01 probability level.

Treatment	Sucrose			Free amino acid		
	Export per leaf[1]	Export per unit FW[2]	Phloem/leaf[3]	Export per leaf	Export per unit FW	Phloem/leaf[4]

	(μg h^{-1} $leaf^{-1}$)	(μg g^{-1} FW h^{-1})	(μg g^{-1} FW h^{-1} /mg g^{-1} DW)	(μg h^{-1} $leaf^{-1}$)	(μg g^{-1} FW h^{-1})	(μg g^{-1} FW h^{-1} /mg g^{-1} FW)
Control	114.7 ± 5.72	46.2 ± 4.05	2.333 ± 0.041	1.203 ± 0.102	0.483 ± 0.070	0.041 ± 0.015
K deficiency	34.7 ± 4.84	12.9 ± 1.23	0.319 ± 0.003	0.761 ± 0.039	0.285 ± 0.026	0.006 ± 0.002
Significance	**	**	**	**	**	**

[1] Exported sucrose or free amino acid per hour per leaf.

[2] Exported sucrose or free amino acid per hour per unit leaf fresh weight.

[3] Ratio of exported sucrose content in phloem exudates to that in leaf per unit leaf dry weight.

[4] Ratio of exported free amino acid content in phloem exudates to that in leaf per unit leaf fresh weight.

Table 2 Effect of K deficiency on pollen viability, pollen tube growth rate, number of ovules per ovary and seed set efficiency.

Treatment	pollen viability (%)	Pollen tube growth rate (μm h^{-1})	Number of ovules per ovary (no. $ovary^{-1}$)	Seed set efficiency (%)
Control	89.0 ± 2.2	110.0 ± 5.7	35.2 ± 1.6	77.8± 3.4
K deficiency	87.6 ± 2.1	65.0 ± 4.9	36.7 ± 2.2	63.0± 3.6
Significance	NS	**	NS	**

** indicate that the differences between two treatments are significant at 0.01 probability levels, respectively. NS means non-significant difference.

Table 3 Correlation coefficients between total soluble carbohydrate content, soluble protein content, ATP content in pistils and pollen tube growth rate as well as seed set efficiency.

Item	Pollen tube growth rate	Seed set efficiency
Total soluble carbohydrates	0.843**	0.864**
Soluble protein content	0.878**	0.817*
ATP	0.925**	0.856**

n = 8, $R_{0.05}$ = 0.707, $R_{0.01}$ = 0.834.

* Significant difference at 0.05 probability level.

** significant difference at 0.01 probability level.