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Influence of potassium deficiency on photosynthesis, chlorophyll content, and chloroplast ultrastructure of cotton plants

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Abstract

In cotton (*Gossypium hirsutum* L.) grown in controlled-environment growth chamber the effects of K deficiency during floral bud development on leaf photosynthesis, contents of chlorophyll (Chl) and nonstructural saccharides, leaf anatomy, chloroplast ultrastructure, and plant dry matter accumulation were studied. After cotton plants received 35-d K-free nutrient solution at the early square stage, net photosynthetic rate (P_N) of the uppermost fully expanded main-stem leaves was only 23 % of the control plants receiving a full K supply. Decreased leaf P_N of K-deficient cotton was mainly associated with dramatically low Chl content, poor chloroplast ultrastructure, and restricted saccharide translocation, rather than limited stomata conductance in K-deficient leaves. Accumulation of sucrose in leaves of K-deficient plants might be associated with reduced entry of sucrose into the transport pool or decreased phloem loading. K deficiency during squaring also dramatically reduced leaf area and dry matter accumulation, and affected assimilate partitioning among plant tissues.

Additional key words: *Gossypium hirsutum*; intercellular CO_2 concentration; net photosynthetic rate; non-structural saccharides; photosynthate translocation; stomata conductance; transpiration rate.

Introduction

Potassium (K) is one of the most important factors influencing crop metabolism, growth, development, and yield. Plant K deficiency resulted in a decrease in P_N (Peaslee and Moss 1968, Longstreth and Nobel 1980, Huber 1984). Deceased P_N of K-deficient leaves has been related to lowered stomata conductance, g_s (Moss and Peaslee 1965, Peaslee and Moss 1968, Raschke 1975), although increased mesophyll resistance may be the primary factor causing the reduction in photosynthesis (Terry and Ulrich 1973, Peoples and Koch 1979, Longstreth and Nobel 1980). K-deficient leaves accumulated soluble sugars and decreased the rate of assimilate export before P_N was reduced (Ashley and Goodson 1972, Mengel 1980). Sucrose transport in K-deficient leaves may be restricted by reduced synthesis of nonstructural saccharides (Conti and Geiger 1982), by reduced sucrose formation (Sugiyama and Goto 1966), by reduced entry of sucrose into the transport pool (Amir and Reinhold 1971), or by inhibition of some steps involved in phloem loading (Mengel 1980, Thompson and Dale 1981, Marschner *et al.* 1996). Lauchli and Pfluger (1978) stressed the role of K as the dominant counterion to light-induced H^+ flux across the thylakoid membranes. In addition, there was a pronounced difference among species in sensitivity of crops to K deficiency (Beringer and Nothdurf 1985).

The application of K fertilizer is important in high-yield cotton production because of the indeterminate growth habit of cotton plants. The occurrence of K deficiency in cotton (*Gossypium hirsutum* L.) has increased across the United States Cotton Belt in recent years (Oosterhuis 1994). Faster fruiting and higher yield cotton cultivars in combination with reduced root growth and ion uptake during reproductive development, and depressed available soil K concentrations may be the major contributors to this increase (Oosterhuis 1994). The sensitivity of cotton to K limitations has led to much research involving soil-applied K (Bennett *et al.* 1965, Kerby and Adams 1985, Cassman *et al.* 1989, 1990, Davis 1996, Pettigrew *et al.* 1996) with the focus of these studies being on yield. Recently, Bednarz and Oosterhuis (1996) investigated the dry matter and K partitioning

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in cotton plant during the development of a K deficiency. However, only a few studies have focused on the physiological implication of K deficiency on cotton plant growth and yield development (Ashley and Goodson 1972, Bednarz and Oosterhuis 1998). A better understanding of the effect of K deficit stress on cotton plant growth and physiological metabolism, especially carbon metabolism, will benefit cotton production by enhancing lint yield and fiber quality. The objectives of this study were to determine the effects of K deficiency during cotton floral bud (square) development on leaf photosynthesis, contents of Chl and nonstructural saccharides, leaf area, dry matter accumulation, leaf anatomy, and chloroplast ultrastructure of growth chamber-grown cotton plants.

Materials and methods

Plants: Seeds of cotton cultivar DP 20 were planted in 4 000 cm^3 pots with washed sand in a growth chamber at the Altheimer Laboratory, University of Arkansas in Fayetteville. The growth chamber was programmed with temperatures of 30/25 °C (day/night), relative humidity of about 50/80 %, a 12-h photoperiod (07:00 –19:00 h), and 580 μmol m^{-2} s^{-1} photosynthetic photon flux density. Seedlings were thinned to one plant in each pot after emergence. Treatments consisted of (*1*) a control with sufficient K supply, and (*2*) K deficiency during the squaring period. All plants were watered every second day with de-ionized water and with modified Hoagland's nutrient solution on alternate days in the first 34 d after planting. When plants reached the early square stage (36 d after planting), K was withheld from the nutrient solution of the K-deficient treatment plants, by substituting 3 mM NH_4NO_3 for 6 mM KNO_3. Control plants continuously received normal nutrient solution containing K.

Measurements: At 35 d after the initiation of the K-deficient treatment when K-deficient symptoms were clearly visible, leaf photosynthetic properties and Chl content were determined. P_N, transpiration rate (E), g_s, leaf temperature (T_l), and intercellular CO_2 concentration (C_i) of the uppermost fully expanded main-stem leaves were recorded at noon with a *LI-6200* portable photosynthesis system (*LI-COR*, Lincoln, NE, USA). Thereafter, six fresh leaf disks, each with an area of 0.38 cm^2, were excised immediately from the same leaves used for P_N measurement to extract Chl using the method of Cornish *et al.* (1991). Chl contents were analyzed spectrophotometrically (Zhao and Oosterhuis 1998).

After sampling leaf disks, the leaves and top stems were rapidly collected, and dried at 90 °C for 30 min, followed by 72 h at 70 °C in preparation for analyzing nonstructural saccharides (hexose, sucrose, and starch). For details of the processes for nonstructural saccharide extraction and measurement see Hendrix (1993) and Zhao and Oosterhuis (1998).

The leaves at the same position on three other plants of each treatment were sampled at 09:00 h to observe leaf anatomy and chloroplast ultrastructure. Leaf tissue specimens, 1-2 mm^2 in size, were prepared for observation with light and transmission electron microscopy. The procedure used by Wullschleger and Oosterhuis (1987) was adopted to fix and dehydrate the tissues. After dehydration, the tissue samples were subsequently embedded in Spurr's epoxy resin, and 1.0-1.5 μm thick sections of leaves were cut with a glass knife for light microscopy observation. The sections were placed on glass slides coated with Haupt's adhesive, stained with 1 % toluidine blue, and photographed on an *Olympus Vanox* microscope. For transmission electron microscopy, thin sections (70-90 nm) were cut with a glass knife, double stained in 2 % uran acetate and lead citrate, and then examined under a *Siemens Elmiskop 1A* transmission electron microscope at 75 kV.

After sampling for saccharide measurement and structural observation, all plants were harvested, and plant height and leaf area were measured. Thereafter, the plant components were dried at 70 °C for 72 h, and the dry masses of leaves, stems, roots, and fruits determined. The unit area leaf mass was calculated by leaf mass divided by leaf area.

Data analysis: The experiment was arranged in a completely randomized design with six replications. Analysis of variance was carried out according to the general linear model procedure of the Statistical Analysis System (*SAS Institute*, Cary, NC, USA). Data means were separated using F-tests, and significant differences were based on p-values ≤ 0.05.

Results and discussion

P_N and Chl content: Leaf P_N on a leaf area basis of the K-deficient plants was only 23 % of the full K supply control plants at 35 d after the removal of K from the nutrient solution (Table 1). Decreased P_N was closely associated with a lower leaf Chl concentration. Total Chl concentration in leaves of K-deficient plants was only 12 % of the control plants with sufficient K. However, there was no difference in the Chl *a/b* ratio between the two treatments, suggesting that contents of both Chl *a* and Chl *b* were reduced synchronously under severe K deficiency. Additionally, K-

deficient cotton leaves [64.9 mmol kg^{-1}(Chl) s^{-1}] had a 2.3-fold higher P_N than the control plants [28.5 mmol kg^{-1}(Chl) s^{-1}]. This suggested that the decrease in P_N of K-deficient plant was probably associated with a reduction in the photosynthesis system rather than its activity. Peoples and Koch (1979) also found that K deficiency did not reduce the activities of photosystem 1, photosystem 2, and ribulose-1,5-bisphosphate carboxylase in alfalfa leaves.

The severe K-deficient cotton plants also had significantly lower leaf g_s, higher T_l, and lower E ($p \leq 0.01$), but higher C_i ($p \leq 0.05$) compared to the control plants (Table 1). These results suggested that K deficiency affected cotton leaf P_N mainly by decreased Chl content, carbon fixation system, and g_s. Bednarz and Oosterhuis (1998) pointed out that during a mild K deficiency increased g_s was the first factor to result in a decrease in leaf P_N, and as the deficiency became more acute, biochemical factors also contributed. Our study also indicated that under severe K deficiency, g_s might not be a main factor decreasing P_N because of higher C_i (Table 1). Therefore, other limitations to P_N, such as the mesophyll resistance, quantity and structure of the photosynthetic reaction center, became dominant.

Table 1. Effects of K deficiency during squaring period on leaf temperature, rates of net photosynthesis (P_N) and transpiration (E), intercellular CO_2 concentration (C_i), stomata conductance (g_s), and chlorophyll (Chl) contents of cotton plants. Measurements were taken in the uppermost full-expanded main-stem leaves, values are the means of six plants. *, **, and *** indicate that the differences between two treatments are significant at 0.05, 0.01, and 0.001 levels, respectively. NS, not significant.

	Control	K deficiency	Significance
Leaf temperature [°C]	30.2	34.2	**
P_N [μmol m^{-2} s^{-1}]	14.0	3.7	***
E [mmol m^{-2} s^{-1}]	9.9	8.8	**
C_i [μmol mol^{-1}]	368	403	*
g_s [cm s^{-1}]	1.11	0.62	**
Chl *a* [mg m^{-2}]	352	41	***
Chl *b* [mg m^{-2}]	139	16	***
Chl (*a*+*b*) [mg m^{-2}]	491	57	***
Chl *a*/*b*	2.53	2.56	NS

Nonstructural saccharides: The maintenance of higher leaf P_N requires that photoassimilates are rapidly transferred from leaves (source) to fruits (sink). Under the K deficiency, the contents of total nonstructural saccharides in cotton leaves increased significantly (Fig. 1*A*), but sucrose, starch, and total nonstructural saccharides of stems (Fig. 1*B*) and floral buds (Fig. 1*C*) were considerably decreased. The contents of hexose, sucrose, starch, and total nonstructural saccharides in leaves of K-deficient cotton plants were 2.9, 4.4, 2.0, and 2.2 fold higher than in the K-sufficient control plants, respectively ($p \leq 0.01$).

An earlier study suggested that accumulation of hexose sugars in K-deficient soybean leaves was associated with increased activities of acid invertase catalyzing sucrose hydrolysis (Huber 1984). But in our study, the amount of increased sucrose in K-deficient cotton leaves was much higher than the amount of increased hexose. K deficiency affected not only the absolute amount of cotton leaf saccharides, but also the components of the sugars. In the control plants, the percentages of leaf hexose, sucrose, and starch accounting for total saccharides were 4.4, 7.7, and 87.9 %, respectively, but they were 5.7, 20.9, and 79.1 %, respectively, in K-deficient plants. In contrast, the stems of K-deficient plants had significantly lower sucrose, starch, and total saccharides contents, except for hexose compared with the control plants. Therefore, K deficiency inhibited the translocation of photosynthates from source to sink through stems, resulting in the accumulation of nonstructural saccharides in leaves. This was a key factor causing the decrease in P_N of K-deficient cotton leaves.

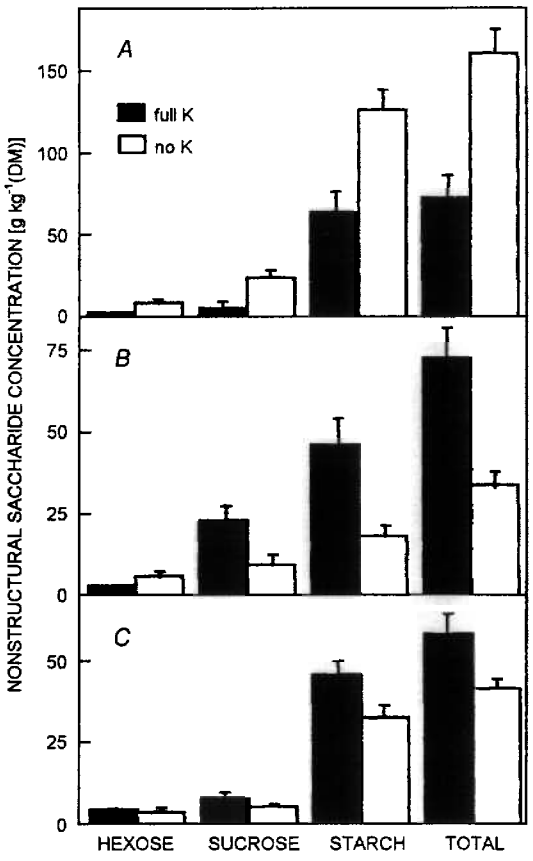

Fig. 1. Comparison of the concentrations of hexose, sucrose, starch, and total saccharides in (*A*) uppermost fully expanded main-stem leaves, (*B*) stems, and (*C*) 15-d-old floral buds of full-K (control) and K-deficient cotton plants. Bars show one side standard error.

Huber (1984) reported that in K-deficient soybean leaves decreased rates of assimilate export were associated with decreased activities of sucrose phosphate synthase, a key enzyme involved in sucrose formation. However, our

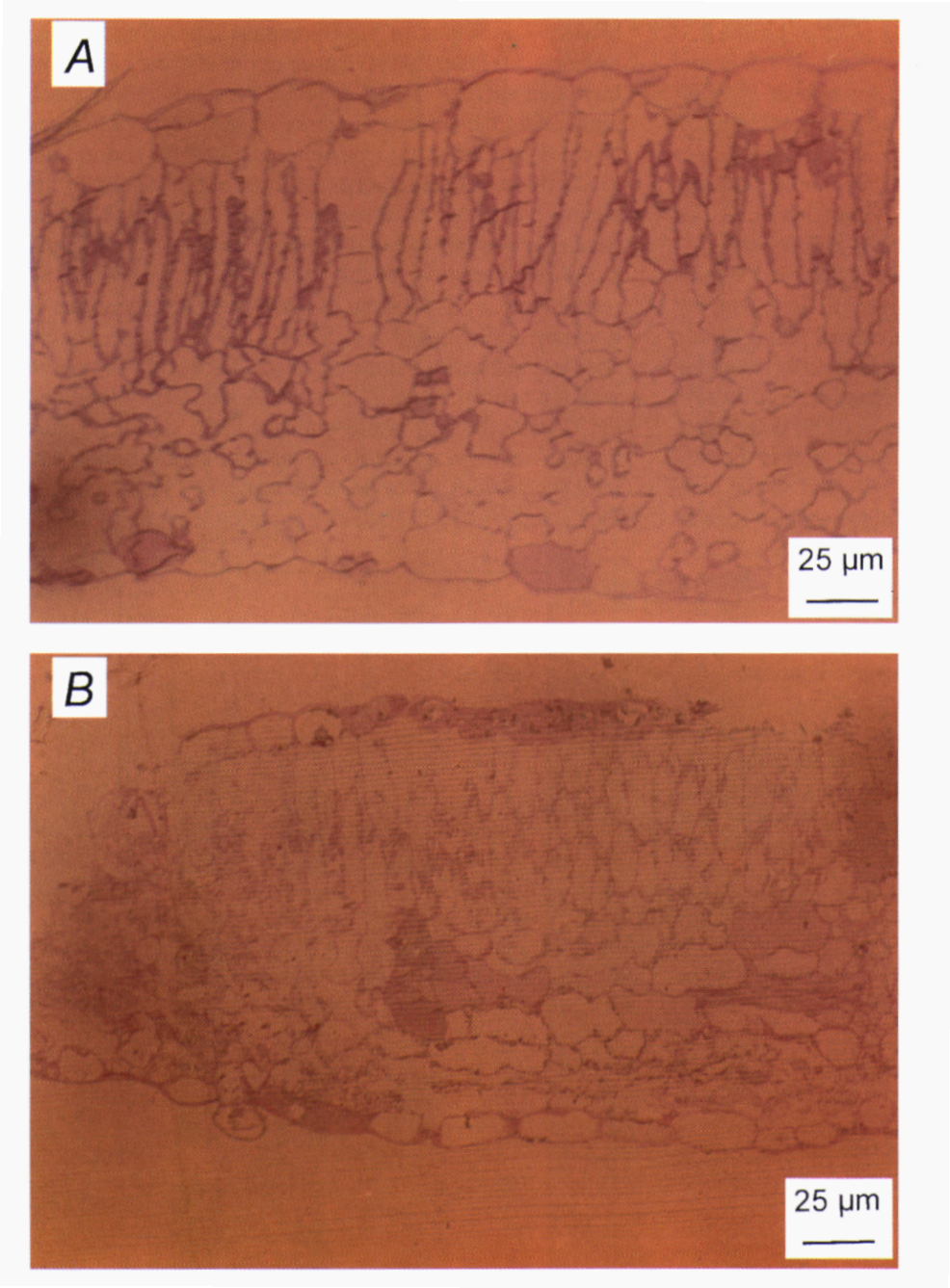

Fig. 2. The cross-sections of leaves in (*A*) K sufficient (control) and (*B*) K deficient cotton plants. The K-deficient leaves had less intercellular space and fewer chloroplasts in the mesophyll cells.

results in cotton plants did not support this conclusion because in K-deficient cotton leaves the increase in sucrose content was much greater than the increase in hexose. Accumulation of sucrose in K-deficient cotton leaves might be associated with reduced entry of sucrose into the transport pool (Amir and Reinhold 1971) or inhibition of some steps involved in phloem loading (Thompson and Dale 1981, Marschner *et al.* 1996), because the content of sucrose in stems of K-deficient cotton plants was pronouncedly lower [9.5 g kg^{-1}(DM)] than in the control plants [23.3 g kg^{-1}(DM)].

Leaf anatomy and chloroplast ultrastructure: Cross-sections of cotton leaves observed with a light microscope indicated that the leaves of K-deficient and control cotton plants had about the same thickness and proportion of palisade cells and spongy mesophyll cells. However, the K-deficient leaf had less intercellular air space and probably fewer chloroplasts in mesophyll cells compared with the control plants (Fig. 2).

A pronounced difference between the control and K-deficient cotton leaves was observed in chloroplast ultrastructure (Fig. 3). The chloroplasts of control plant

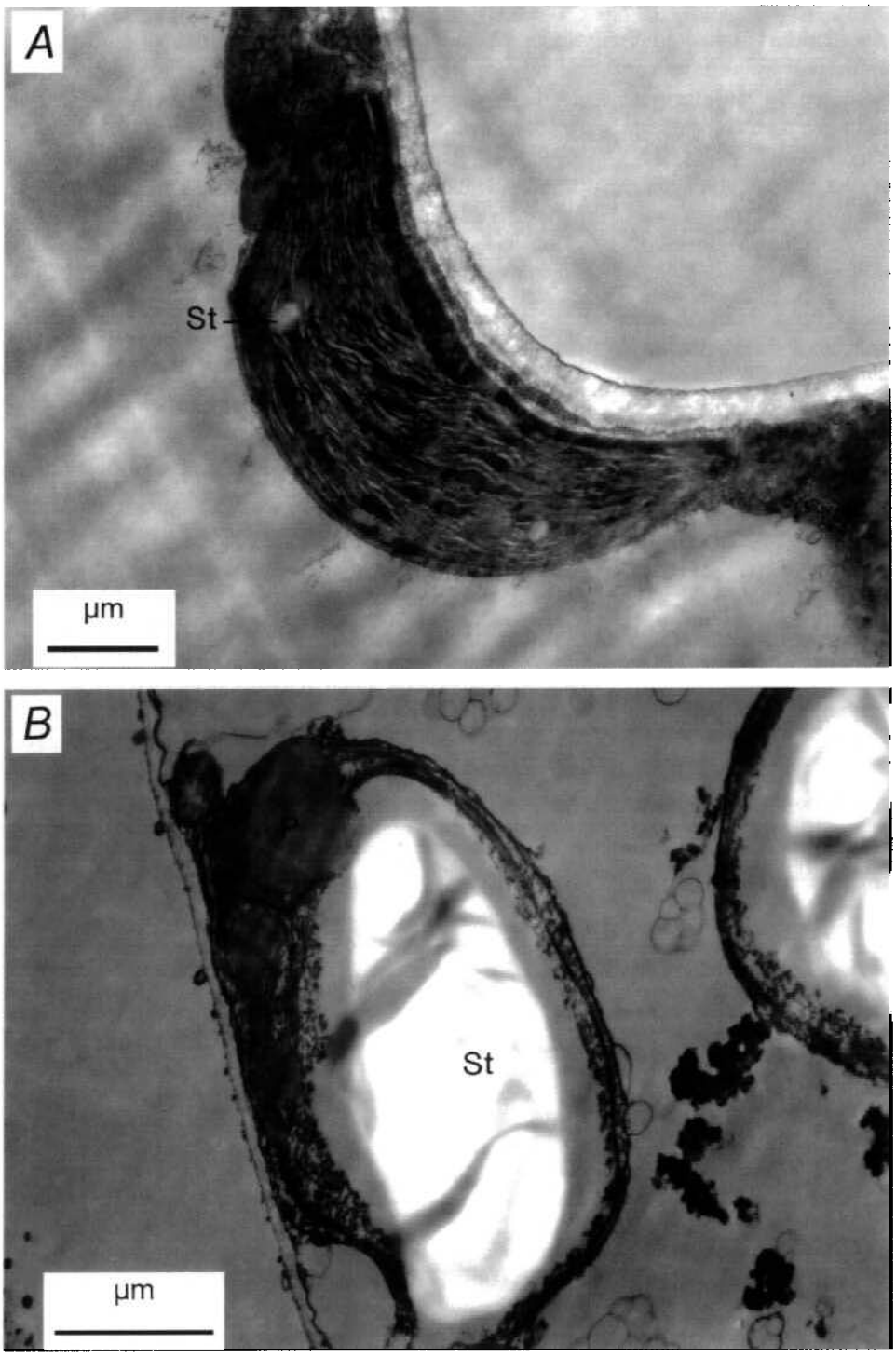

Fig. 3. Comparison of the chloroplast ultrastructures of (*A*) K-sufficient (control) and (*B*) K-deficient cotton leaves. *St*: starch granule; *G*: grana; *P*: plastoglobuli.

leaves had more well-defined grana stacks and extensive stroma lamellae with very small amounts of starch granules (Fig. 3*A*). The chloroplasts in leaves of K-deficient plants were, on the other hand, filled with large starch granules, and contained markedly more and greater plastoglobuli and fewer grana (Fig. 3*B*). A similar result was found in K-deficient maize by Hall *et al.* (1972). The leaves were sampled early morning (at 09:00 h) in our study. All plants only received light period of two hours at that time. The photosynthates in the leaves of the control plants were translocated to young bolls, squares, and other sinks during the night period. Inhibited translocation of photosynthates resulted in starch accumulation in the chloroplasts of leaves of K-deficient plants. The conclusion that K deficiency

decreased the translocation of saccharides from cotton leaves to fruits was further supported by the starch accumulation in the chloroplasts shown by the electron microscope observations. In addition, the poor chloroplast ultrastructure, including starch granule accumulation, low Chl content, fewer grana, disorientation of grana and thylakoids that occurred as they were pushed towards the periphery of the chloroplast, might also be one of causes of low leaf P_N in K-deficient cotton.

Table 2. Comparison on plant height, leaf area, dry masses of stems, leaves, roots, and fruits, and unit area leaf mass (UALM) after a 35-d K-deficient treatment during square development. Means of six plants. *, **, and *** indicate that the differences between two treatments are significant at 0.05, 0.01, and 0.001 levels, respectively.

Treatment	Control	K deficiency	Significance
Plant height [cm]	72	51	*
Leaf area [cm^2 $plant^{-1}$]	3798	1773	**
Stem mass [g $plant^{-1}$]	14.53	6.44	***
Leaf mass [g $plant^{-1}$]	16.90	10.16	**
Root mass [g $plant^{-1}$]	11.37	5.11	***
Fruit mass [g $plant^{-1}$]	5.24	2.01	**
Total mass [g $plant^{-1}$]	48.04	23.72	***
UALM [g m^{-2}]	44.50	57.30	*

Leaf area and dry matter accumulation: K deficiency during cotton square development significantly decreased plant height, leaf area, and dry matter accumulation (Table 2). Plant height, leaf area, and total dry mass of the K-deficient cotton in this study decreased by 29, 53, and 51 %, respectively, compared to full K supply control plants. However, the different plant parts exhibited a differential range of decline in dry masses, with the magnitude of decreased percentage of dry mass being in the order of fruit (62 %) > stem (56 %), or root (55 %) > leaf (40 %). These results suggest that severe K deficiency during square development caused the greatest decrease in fruit dry mass, and the least decrease in leaf dry mass. K-deficient cotton plants had significantly higher unit area leaf mass (UALM) than the control plants (29 % increase). Increased UALM was not attributed to a thicker leaf, but to less intercellular space and higher nonstructural saccharide accumulation in leaves, as shown by the leaf anatomy, chloroplast ultrastructure, and saccharide contents of K-deficient plants. Decreased fruit dry mass for the K-deficient plants was closely related to both a decrease in fruiting sites and an increase in fruit shedding. Insufficient saccharose supply was a major factor causing fruit shedding of K-deficient plants.

Conclusion: K deficiency during cotton square development significantly restricted the translocation of photosynthates from leaves to fruits, increased contents of hexose, sucrose, and starch in leaves, decreased sucrose and starch contents in stems and floral buds. This led to dramatically lower Chl content and the poor development of leaf anatomy and chloroplast ultrastructure. As a result, P_N per unit leaf area, leaf area and dry matter production, especially fruit dry matter accumulation, decreased significantly compared to the control plants of sufficient K supply. These findings further our current understanding of cotton potassium use and should be useful for improving K fertilizer management in cotton production.

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