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Research article

Potassium deficiency affects the carbon-nitrogen balance in cotton leaves

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ABSTRACT

Potassium (K) plays important roles in the metabolism of carbon (C) and nitrogen (N), but studies of K deficiency affecting C-N balance are lacking. This study explored the influence of K deficiency on C-N interaction in cotton leaves by conducting a field experiment with cotton cultivar DP0912 under two K rates (K0: 0 kg K_2O ha^{-1} and K67: 67 kg K_2O ha^{-1}) and a controlled environment experiment with K-deficient solution (K1: 0 mM K^+) and K-sufficient solution (K2: 6 mM K^+). The results showed that leaf K content, leaf number, leaf area, boll number, reproductive dry weight and total dry weight were significant lower under K deficiency (K0 or K1). Lower total chlorophyll content and Chl a/b ratio, and decreased *Pn* along with lower *Gs* and higher *Ci* were measured under K deficiency, suggesting that the decrease in *Pn* was resulted from non-stomatal limitation. Leaf glucose, fructose, sucrose and starch contents were higher under K deficiency, because lower sucrose export was detected in phloem. Although leaf nitrate and ammonium contents significantly decreased, free amino acid content was increased by 40–63% under K deficiency, since lower amino acid export was also measured in phloem. K deficiency also induced lower soluble protein content in leaves. Leaf ATP level was significantly increased under K deficiency, indicating ATP utilization was lower, so that less energy was supplied to C and N metabolism. The ratio of soluble sugar to free amino acid and the C/N ratio markedly increased under K deficiency, and one reason was that the phloem export reduced more prominent for sucrose (54.6–78.0%) than amino acid (36.7–85.4%) under K deficiency. In addition, lower phosphoenolpyruvate carboxylase activity limited malate and citrate biosynthesis under K deficiency, causing a decrease of C flux into the amino acids, which was not beneficial for maintaining C-N balance. Sucrose phosphate synthase and nitrate reductase activities were lower under K deficiency, which would limit sucrose biosynthesis and nitrate assimilation. This was another factor altering soluble sugar to free amino acid ratio and C/N ratio in the K-deficient leaves.

1. Introduction

Potassium (K) is important for ensuring optimal plant growth. Although K is not a constituent of any tissue in plants, it is the most abundant inorganic cation, comprising up to 10% of a plant's dry weight (White and Karley, 2010). K plays important roles in numerous physiological and metabolic processes, like maintenance of transmembrane voltage gradients, cation-anion balance (White and Karley, 2010), osmotic potential and water uptake (Kaiser, 1982), regulating the movement of stomata (Humble and Raschke, 1971) and activation of enzymes (Evans and Sorger, 1966). Investigators also reported that K is needed for CO_2 assimilation (Hu et al., 2015) and nitrogen (N) assimilation (Drosdoff et al., 1947).

Cotton (*Gossypium hirsutum* L.) has a higher demand for K to

Abbreviations: PEPCase, phosphoenolpyruvate carboxylase; SPS, sucrose phosphate synthase; NR, nitrate reductase; ATP, adenosine triphosphate; *Pn*, net photosynthetic rate; *Gs*, stomatal conductance; *Ci*, intercellular CO_2 concentration; FW, fresh weight; DW, dry weight; SLW, specific leaf weight.

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maintain plant growth and fiber development than other crops with determinate growth habits. Many investigators reported that K deficiency resulted in low seed cotton yield and lint yield (Pettigrew, 1999), due to less boll number (Li et al., 2012), lower boll weight (Gormus, 2002) and lower lint percentage (Pettigrew, 1999). K deficiency negatively affected cotton fiber qualities including fiber length, uniformity ratio, fiber strength, and micronaire (Pettigrew et al., 2005). Some studies also indicated that K deficiency would alter biomass accumulation and partitioning (Makhdum et al., 2007) and morphological indices (Gerardeaux et al., 2009). K deficiency also affected numerous metabolic processes, such as carbon (C) metabolism and N metabolism. Zhao et al. (2001) found that K deficiency could alter the contents of sucrose and starch in leaves, and the percentages of sucrose and starch accounting for total carbohydrates. The activities of Rubisco related to CO_2 assimilation and cy-FBPase involved in the first step of sucrose synthesis were markedly reduced by K deficiency (Hu et al., 2015). Drosdoff et al. (1947) reported that K^+ was necessary for N metabolism in plants, because NO_3^- was transported together with K^+ in the xylem (Dong et al., 2004). Hu et al. (2016b) also observed that K deficiency reduced NO_3^- allocation to the subtending leaves of cotton. Thus, K deficiency affected the C and N metabolism in plants. However, a comprehensive understanding of the effects of K deficiency on C-N interaction is lacking.

Carbon metabolism and N metabolism are linked because they share organic C and energy supplied by photosynthetic electron transport, CO_2 fixation or respiration (Huppe and Turpin, 1994). As a consequence, there are strong interactions between C assimilation and N assimilation in metabolic processes and energy levels (Fait et al., 2011). Between C assimilation and N assimilation, the oxaloacetate-malate shuttle system serves as a valve regulation the reduction of CO_2 and NO_2^-, and malate content was closely linked to CO_2 assimilation and NO_2^- reduction (Backhausen et al., 1994). Champigny (1995) observed that three enzymes (PEPCase, phosphoenolpyruvate carboxylase; SPS, sucrose phosphate synthase; NR, nitrate reductase) play crucial roles in the C-N interaction. In addition, an interaction between C and N metabolites is observed, because the loading of amino acids depends on sucrose loading and mass flow in the phloem (Wang et al., 2012), and there is a fixed ratio of sucrose to amino acids in the cytosol of phloem (Cakmak et al., 1994). However, reports of the effects of K deficiency on the interaction between sucrose transport and amino acid transport in phloem are lacking.

Therefore, it was hypothesized that K deficiency would influence C/N balance in cotton leaves and change the export ratio of sucrose to amino acid in phloem. The objectives of this study were (1) to explore the effects of K deficiency on C metabolism, N metabolism and C/N balance in cotton leaves in more detail, and (2) to investigate the effects of K deficiency on the export of C and N metabolites in phloem and its relationship with C/N balance in leaves.

2. Materials and methods

2.1. Experiment design

2.1.1. Field study

A field experiment was arranged at the Lon Mann Cotton Research Station in Marianna, AR (34°5′N, 90°5′W) in the summer season of 2015. The available K content in soil before sowing was 72.7 mg kg^{-1} which was below levels needed for optimal cotton growth (Oosterhuis, 2002). The seeds were sowed on May 14 in Marianna. The cotton cultivar DP 0912 was selected and a randomized complete block was arranged with four replications. Two K fertilizer levels (K0: 0 kg K_2O ha^{-1} and K67: 67 kg K_2O ha^{-1}) were applied at the beginning of flowering stage in reference to our former study (Oosterhuis et al., 2014a,b). Each plot size was 4 m × 15 m with 1 m row spacing, and the plant density was 74,000 plants ha^{-1}. Weed and insect control was conducted as needed and furrow irrigation was applied according to the Arkansas irrigation scheduler program, which is based on soil moisture balance and evapotranspiration.

2.1.2. Greenhouse study

A controlled environment (greenhouse) experiment was established at the Altheimer Laboratory, University of Arkansas. The same cultivar was planted on January 20, 2015 in 2-L pots in two same growth chambers (Conviron PGW36, Conviron Inc., Winnipeg, Manitoba, Canada). The growth chambers were set for a 12/12 h photoperiod, a photosynthetic flux density of 800–850 μmol m^{-2} s^{-1}, a relative humidity of 60% and temperatures of 30/25 °C (day/night). Each growth chamber was arranged with 24 pots and each pot just had one plant. One of the growth chambers was regarded as an experiment repeated. The Hoagland's nutrient solution contained 6 mM K^+, 2 mM NH_4^+, 4 mM Ca_2^+, 2 mM Mg_2^+, 1 mM Fe^{3+}, 3.7 μM Mn^{2+}, 0.77 μM Zn^{2+}, 0.32 μM Cu^{2+}, 7.3 μM Cl^-, 2 mM PO_4^{3-}, 2 mM SO_4^{2-}, 46 μM H_3BO_3 and 0.12 μM MoO_3, and all pots were watered every two days with one-quarter-strength K nutrient solution (1/4 strength K concentration in above Hoagland's nutrient solution through substituting NH_4NO_3 for KNO_3) and with deionized water alternately until flowering. Two treatments were established at the beginning of flowering stage, containing (1) a treatment without K in the nutrient solution (K1: 0 mM K^+), and (2) a control with sufficient K supply (K2: 6 mM K^+). Pots were re-randomized once a week in each chamber from seed germination to the end of the experiment.

2.2. Sampling and processing

At 4 weeks after first flower (90 days after sowing, August 12), the plants in the K0 treatment have showed severe K deficiency symptoms. Four leaves at the fourth main-stem node from the apex of the plant in each plot in the field experiment were used for the measurement of photosynthetic parameters, then were sampled for the measurement of chlorophyll content by removing five discs (0.75 cm^2 per disc) in 80% acetone extracts (Lichtenthaler, 1987), then the leaves with petiole were transported on ice to the lab for the analyses of leaf K content, carbohydrates and N compounds. At 6 weeks after first flower (104 days after sowing, August 26), agronomic traits (height, fruiting branch number, leaf number, leaf area, boll number) were measured. Leaf area was recorded by a LI-3100 area meter (LiCor, Linoln, NE, USA). The plants above ground collected from one-meter row in each plot were divided into stems (and petioles), leaves, and reproductive organs. Dry matter weights of these parts were recorded after drying at 80 °C for 72 h.

In the greenhouse experiment, at 4 weeks after first flower (80 days after sowing, April 10) four leaves at the fourth main-stem node from the apex of the plant were used for the measurement of photosynthetic parameters and chlorophyll content. Eight leaves were sampled for leaf K content, carbohydrates, N compounds and enzymes determinations. Four leaves were used for collecting phloem exudates. Agronomic traits and dry matter weight of plants were also measured with four replications at 6 weeks after first flower (94 days after sowing, April 24).

2.3. Photosynthetic parameters

Before sampling the leaves at the fourth main-stem node from the terminal of the plant, photosynthetic parameters including net photosynthetic rate (*Pn*), stomatal conductance (*Gs*) and