Field Crops Research 214 (2017) 301–309

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

Cultivar sensitivity of cotton seed yield to potassium availability is associated with differences in carbohydrate metabolism in the developing embryo

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ARTICLE INFO

Keywords:
Potassium
Cottonseed
Carbohydrate metabolism

ABSTRACT

Storage lipids and proteins in cottonseed have important industrial values and carbohydrate metabolism is the basis for the biosynthesis of oil and protein in cottonseed. In order to investigate the effects of potassium (K) fertilizer on carbohydrate metabolism of cottonseeds in two cotton (*Gossypium hirsutum* L.) cultivars with different K sensitivities, a two-year field experiment was conducted with a low K-tolerant cultivar (Simian 3) and a low K-sensitive cultivar (Siza 3) under three K levels (0, 150 and 300 kg K_2O ha^{-1}). Results showed that boll number and seed index were higher in the K application treatments (150 and 300 kg K_2O ha^{-1}) than in the 0 kg K_2O ha^{-1}, resulting in high cottonseed yield. Embryo weight was increased by K application, but seed coat weight was not influenced. K application did not change protein content, but markedly increased oil and non-structural saccharide contents, and K concentration was positively correlated with oil and non-structural carbohydrate contents. In addition, higher non-structural carbohydrate content in the K application treatments than in the 0 kg K_2O ha^{-1} was attributed to higher contents of starch, sucrose and fructose, and sucrose increased to a greater extent than other carbohydrates with K application. Higher fructose content in the K application treatments was closely related to higher sucrose synthase (SuSy) and acid invertase activities. Compared with Simian 3, the sensitivity of Siza 3 to K was evidenced in the following ways: (1) boll number, cottonseed yield, embryo biomass, and the seed coat to embryo ratio were increased more by K application for Siza 3 than Simian 3; (2) oil and carbohydrate accumulations in embryo were more responsive to K concentration in Siza 3 than Simian 3; (3) the increases in the contents of sucrose, starch and fructose and the activities of enzymes (SuSy and acid invertase) caused by K application were larger in Siza 3 than Simian 3; (4) the increases in sucrose phosphate synthase and alkaline invertase activities resulting from K application was only detected in Siza 3.

1. Introduction

Cotton (*Gossypium hirsutum* L.) not only is the main fiber crop globally, but also is the second largest potential source of plant protein and the fifth largest oil-producing plant in the world (Sawan et al., 2006; Hu et al., 2017). Cottonseed is the main byproduct of cotton production and contains approximately 20% oil and 20% protein by weight, which are used for ruminant feed, cooking, and renewable biofuels (Chen et al., 2015). Yield and quality of cottonseed are very sensitive to changes in environment (Chen et al., 2015). Some studies have revealed that many abiotic stresses could influence cottonseed yield and quality, such as low nitrogen stress (Sawan et al., 2006), drought stress (Chen et al., 2015) and low temperature stress (Hu et al., 2017). Potassium (K) as a macronutrient plays a key role in physiological processes of plants, but the effects of K on cottonseed yield and quality are lacking.

Compared with other crops, cotton is more sensitive to low K conditions because of its indeterminate growth habit (Oosterhuis, 2001). In addition, different cotton plant organs exhibit different sensitivities to K deficiency, with the order of sensitivity being fruits > stems > roots > leaves (Dong et al., 2004). In cotton fibers, K is one of the main osmotically active solutes that produces the turgor pressure essential for cotton fiber elongation (Dhindsa et al., 1975). K also affects the translocation of sugars from the leaf to the fiber (Oosterhuis and Bednarz,

Abbreviations: DAA, days after anthesis; SuSy, sucrose synthetase; SPS, sucrose phosphate synthase

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http://dx.doi.org/10.1016/j.fcr.2017.09.022
Received 6 August 2017; Received in revised form 15 September 2017; Accepted 18 September 2017

1997), which influences secondary cell wall deposition in developing fiber cells. Thus, K deficiency has some negative effects on fiber strength (Minton and Ebelhar, 1991) and micronaire (Pettigrew et al., 2005). In leaves, past studies have shown that K deficiency significantly reduced photosynthetic rates of individual leaves (Bednarz and Oosterhuis, 1999; Zhao et al., 2001) and canopies (Reddy et al., 2000) of cotton. K is also required for carbohydrate and nitrogen metabolisms in cotton leaves. In the process of carbon assimilation, sucrose and starch contents in cotton leaves increased under K deficiency (Bednarz and Oosterhuis, 1999), and Rubisco and sucrose phosphate synthase (SPS) (Hu et al., 2015, 2016a) activities were decreased by K deficiency. In the process of nitrogen assimilation, K deficiency altered the distribution of nitrogenous compounds between amino acid and protein, and decreased NR activity (Hu et al., 2016d). In cottonseed, K fertilizer application could alter oil yield and protein yield (Sawan et al., 2006) and change oil properties (Sawan et al., 2007). Lipids are synthesized in several different sub-cellular organelles using carbon skeletons provided by sugars imported from leaves, and seed storage proteins are synthesized using amino acids imported directly from source organs, or from transamination reactions involving carbon skeletons in the sink organs (Liu et al., 2012). Thus, carbon skeleton availability is closely related to oil and protein biosynthesis in the seed, and the changes in oil and protein accumulation caused by K application may be related to altered carbohydrate metabolism. However, studies of the effects of K nutrition on carbohydrate metabolism in the cottonseed are lacking although these effects have been investigated in cotton leaves (Hu et al., 2016a) and fibers (Yang et al., 2016).

Genotypic differences in K sensitivity have been reported in various crops (Jia et al., 2008; Li et al., 2011), including cotton (Zhang et al., 2007). Cotton cultivars with different K sensitivities were screened out using pot and field experiments previously (Tian et al., 2008; Yang et al., 2014), and different cotton cultivars had different physiological responses to K deficiency. Wang et al. (2012) reported that the low K-sensitive cotton cultivar had lower carbon assimilation in leaves under K deficiency relative to the low K-tolerant cultivar. Yang et al. (2016) also reported that K availability has different effects on sucrose and cellulose metabolism in cotton fibers between the low K-tolerant cultivar and the low K-sensitive cultivar. Therefore, it is speculated that K treatment will also differentially affect carbohydrate metabolism of cottonseed in tolerant and sensitive genotypes of cotton. The objectives of this study were (1) to explore the effects of K application on carbohydrate metabolism, growth, and development of the cottonseed, and (2) to identify the differences in cottonseed carbohydrate metabolism of a low K-tolerant cultivar and a low K-sensitive cultivar in response to K availability. This work could be useful in identifying the mechanisms contributing to high cottonseed quality in genotypes that are tolerant to K deficiency.

2. Materials and methods

2.1. Experimental design

A two-year field experiment was established using two cotton cultivars with different K sensitivities (Simian 3, low K-tolerant cultivar; Siza 3, low K-sensitive cultivar) screened from previous experiment (Yang et al., 2014) at the Pailou Experimental Station of Nanjing, China (118°50′E, 32°02′N). The experiments were carried out in two different parts of the same field for 2012 and 2013 in order to avoid residual K fertilizer effects in the soil from the previous year's experiment. The type of the soil at the experimental site was clay, mixed, thermic, Typic alfisols (udalfs; FAO luvisol) with a pH of 6.7. The soil (0–20 cm) before sowing contained 15.9 and 17.1 g kg^{-1} organic matter, 69.8 and 77.3 mg kg^{-1} alkali-hydrolyzable N, 23.6 and 18.1 mg kg^{-1} available P, and 86.3 and 91.8 mg kg^{-1} available K in 2012 and 2013, respectively. Seeds were planted in a nursery bed on 23 April 2012 and on 30 April 2013, and cotton seedlings with three true leaves were transplanted into the experimental field on 23 May 2012 and on 30 May 2013. The experiment was arranged as a two-factor split plot design with three replications. The main plots were varieties (Simian 3 and Siza 3) and subplots were K levels (0, 150 and 300 kg K_2O ha^{-1} using potassium sulphate at the transplanting stage). Each plot was consisted of fifteen rows, and the plot size was 13 × 6.6 m^2 with 0.85 m row spacing and 0.35 m interplant spacing within a row. The plant density was 3.4 plants m^{-2}. In order to avoid the effects of different sulfur levels on cotton growth and development, enough ordinary superphosphate (12% P_2O_5 and 12% sulfur) was applied at the transplanting stage to provide P fertilizer of 120 kg ha^{-1}. 240 kg N ha^{-1} was applied using urea, and 40% of urea was applied at the transplanting stage and 60% of urea was applied at the flowering stage.

2.2. Sampling and processing

White flowers at the first fruiting position of fruiting branches at the 7th or 8th mainstem node attachment were tagged to indicate the flowering date and calculate the days after anthesis (DAA) of sampled bolls. These tagged bolls were sampled at 17, 31 and 45 DAA from 9:00-10:00 h 4–6 cotton bolls in each plot and all cottonseeds in each boll were collected at each sampling time. The sampled cottonseeds were divided into two halves. After the cottonseed coat was removed from the embryo, one half of the cotton seed was immediately placed in liquid nitrogen and stored at −80 °C for enzyme measurements in the embryo, and the other half was used to determine carbohydrate contents. At 45 DAA, the embryos were also used for the determinations of oil content, protein content, non-structural carbohydrate content (hexose, sucrose and starch) and K concentration measured from a H_2SO_4−H_2O_2 digestion solution of each sample by an atomic absorption spectrophotometer (SpectAA-50/55, Varian, Australia). When the bolls opened, boll number was recorded and 50 bolls in each plot were harvested for the assessment of seed number per boll, seed index, seed coat weight, embryo weight and cottonseed yield. Cottonseed yield was calculated as boll number × seed number per boll × seed index/(100×10^3).

2.3. Measurement of embryo oil and protein contents

Embryo oil content was determined using the method of Soxhlet extraction (Luque de Castro and Garcıa-Ayuso, 1998). The N concentration of the embryo was measured using the Kjeldahl method (Feil et al., 2005), and embryo protein content was calculated as 6.25 × N concentration (Chen et al., 2015).

2.4. Measurement of carbohydrate contents

Embryo samples used for carbohydrate analysis were dried at 70 °C for 3 days in a forced air oven then a ground (homogenized) embryo sample (0.1 g) was put into 5 mL of 80% (v/v) ethanol and incubated for 30 min at 80 °C. Next, the mixture was centrifuged at 4000*g* for 5 min. The above process was repeated two more times with the liquid portion being decanted each time following centrifugation. All supernatants were combined and diluted with 80% ethanol to a final volume of 25 mL. The resulting extract was used for the measurement of glucose, fructose and sucrose according to Hendrix (1993).

The residue was used for starch extraction according to Hu et al. (2015). After ethanol was evaporated, the residue was heated at 100 °C for 15 min along with 2 mL distilled water. Then, 2 mL of 9.2 mol L^{-1} $HClO_4$ was added. After15 min, 4 mL distilled water was added. The samples were centrifuged at 4000*g* for 10 min. The residue was extracted again with 4.6 mol L^{-1} $HClO_4$ (2 mL). The two supernatants were collected and diluted with distilled water to a total volume of 25 mL. The starch concentration was assayed using an anthrone reagent and the solution absorbance was measured at 620 nm as described previously (Wang et al., 2016).