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The variability of cottonseed yield under different potassium levels is associated with the changed oil metabolism in embryo

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ABSTRACT

Although the effects of potassium (K) fertilizer on cottonseed yield have been explored, reports on cottonseed yield components and related oil metabolism in genotypes differing in sensitivity to low K are limited. To this end, the cultivars Simian 3 (low K-tolerant) and Siza 3 (low K-sensitive) were grown in 2012 and 2013 under three K_2O rates (0, 150 and 300 kg ha^{-1}). Results indicated that K application treatments (150 and 300 kg K_2O ha^{-1}) increased seedcotton yield, oil yield, protein yield, seed weight boll^{-1} and individual seed weight. Higher individual seed weight as the most basic cottonseed yield component was attributed to the enhanced oil weight seed^{-1} rather than protein weight seed^{-1} at higher K application rates. Higher oil weight seed^{-1} in the K application treatments than the 0 kg K_2O ha^{-1} treatment was a consequence of differences in oil accumulation dynamics. Additionally, higher rates of oil accumulation in the K application treatments were closely associated with phosphatidic acid phosphatase and glucose-6-phosphate dehydrogenase activities. Fatty acid composition was also altered by K treatment, where the percentage of palmitic and tetradecanoic acids decreased and linoleic acid percentage increased with K application, leading to a higher ratio of total unsaturated fatty acids to total saturated fatty acids. Furthermore, the magnitudes of increases in seedcotton yield and individual seed weight with K application were greater in the low-K sensitive cultivar than the low-K tolerant one. K application influenced oil accumulation dynamics and fatty acid saturation levels to a greater extent in the low-K sensitive cultivar than the low-K tolerant cultivar. In conclusion, K application could positively influence cottonseed yield by increasing oil accumulation in individual seed and positively improve the unsaturated fatty acid percentage of cottonseed oil.

1. Introduction

Although cotton (*Gossypum hirsutum* L.) is primarily grown for fiber, cottonseed has tremendous value as well. For example, total cottonseed yield globally was 4200–4500 million tons per year (Gao et al., 2014), and per hundredweight of seed fetched 20.15 dollars by selling directly (Coppess et al., 2017). Cottonseed is high in oil and protein, which makes cotton the second largest potential source of plant protein after soybean (*Glycine max* L.) and the fourth largest oil crop after soybean, rape (*Brassica napus* L.) and palm (*Elaeis guineensis* L.) (Thirumalaisamy et al., 2016). Even in some countries, such as Pakistan and India where soybean yield is not very high, cottonseed is the main source of vegetable oil (Chaudhry, 2008). Therefore, maintaining high cottonseed yield is critical. Cottonseed is one of the most important sinks for the cotton plant, and a large amount of photosynthate is transported to cottonseed during seed maturation and fiber growth, which occur concomitantly. During the seed maturation period, carbon skeletons are mainly utilized to synthesize storage oil and protein (Snider and Oosterhuis, 2015), where total storage lipid and protein contents of a mature embryo are up to 80% (Liu et al., 2012). Thus, the process of cottonseed growth mainly involves the accumulation of oil and protein.

Previous studies have reported that abiotic stresses, such as water stress (Zhu et al., 2018), high salinity (El-Beltagi et al., 2017) and nutrient deficiency (Sawan et al., 2001), could significantly decrease cottonseed yield. Potassium (K) plays an indispensable role in cotton growth (Oosterhuis et al., 2013), and K deficiency reduces seedcotton

Abbreviations: DPA, days post anthesis; PK, pyruvate kinase; G6PDH, glucose-6-phosphate dehydrogenase; PPase, phosphatidic acid phosphatase; W_m, the theoretical maximum oil concentration of the embryo; T, the duration of the rapid oil increase phase; V_m, the maximum rate of oil increase; t_m, the occurrence time of V_m; V_T, the average increase rate of oil concentration; TU, total unsaturated fatty acids; TS, total saturated fatty acids

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yield, lint yield, boll biomass and boll weight (Pettigrew et al., 2005; Hu et al., 2016b). Sawan et al. (2006b) also reported that K deficiency could decrease final cottonseed yield per unit due to unfavorable effects of K deficiency on yield components such as number of bolls, seed number boll^{-1} and seed index (100-cottonseed weight) (Pervez et al., 2005; Sawan et al., 2007a). Individual seed weight as the most basic cottonseed yield component increases during development follow a sigmoidal growth curve (Li et al., 2009), and the accumulation pattern of seed oil closely parallels the pattern of seed weight accumulation in a number of oilseeds, such as *Arabidopsis* (Focks and Benning, 1998; Baud et al., 2002), rape seed (Eastmond and Rawsthorne, 2000) and soybean seed (Yazdi-Samadi et al., 1977). Results reported by Sawan et al. (2006a) and Song et al. (2015) also indicated that seed index should be closely tied to total seed oil concentration at harvest. However, those studies have not addressed the effects of K application on the process of seed oil accumulation.

Lipid accumulation is a complicated process in plant seeds. The direct precursor of fatty acid synthesis is acetyl-CoA, and the level of acetyl-CoA can influence the rate of fatty acid synthesis. Phosphoenolpyruvate (PEP) is dephosphorylated by pyruvate kinase (PK) to generate pyruvate and ATP, and pyruvate is decarboxylated and converted to acetyl-CoA. Thus, PK plays a key role in acetyl-CoA synthesis (Baud et al., 2007). Fatty acid synthesis is energy demanding since a fixed number of ATP and NADPH are needed for the addition of each acetyl unit to an elongating fatty acid chain (Baud and Lepiniec, 2010). Schwender et al. (2003) have demonstrated that the oxidative pentose phosphate pathway can produce up to 60% of the NADPH for the biosynthesis of fatty acids, and glucose-6-phosphate dehydrogenase (G6PDH) is one of the key enzymes for generating NADPH in this pathway. Triglycerides are the main form of storage oils and diacylglycerol is the immediate precursor of triacylglycerol synthesis (Baud and Lepiniec, 2010). Phosphatidic acid phosphatase (PPase) dephosphorylates phosphatidate to yield diacylglycerol, hence PPase is also important for the synthesis of triglycerides in seeds (Nakamura and Ohta, 2010). Previous reports have demonstrated that K application could increase final cottonseed oil content (Swan el., 2007b; Song et al., 2015), but studies demonstrating how K influences the aforementioned physiological processes involved in oil synthesis in cottonseeds are limited.

Many crops exhibit genotypic differences in K sensitivity, including rice (*Oryza sativa* L.) (Jia et al., 2008), potato (*Solanum tuberosum* L.) (Trehan and Sharma, 2002), and cotton (Zhang et al., 2007). Previous studies have documented that carbon and nitrogen assimilation responses in leaves to K application vary based on cultivar differences in K sensitivity (Wang et al., 2012; Hu et al., 2016c). Similarly, cotton cultivars have been shown previously to differ in sensitivity of fiber cellulose accumulation (Yang et al., 2016) and carbohydrate metabolism in developing cottonseeds to K application rates (Hu et al., 2017a). Because carbohydrates are the basis for oil biosynthesis in seeds (Liu et al., 2012), lipid accumulation dynamics and the activity of enzymes involved in lipid biosynthesis in seeds should exhibit cultivar-specific responses to K application.

Therefore, our experiment was conducted to test the hypotheses that K application have significant influences on cottonseed yield components (individual seed weight etc.), oil accumulation and related physiological processes, and (2) that two cultivars with known differences in K sensitivity will exhibit significant differences in yield components and oil accumulation dynamics in developing embryos.

2. Materials and methods

2.1. Plant material and sampling method

The cotton cultivars Simian 3 (a low K-tolerant cultivar) and Siza 3 (a low K-sensitive cultivar), identified from our previous experiment (Yang et al., 2014), were planted in an experimental field in 2012 and 2013 at the Pailou Experimental Base, Nanjing Agricultural University, China (118°50′E, 32°02′N). The soil was clay (pH 6.7) with available K content of 86.3 and 91.8 mg kg^{-1} (0–20 cm) in 2012 and 2013, respectively, which is considered insufficient for cotton growth at this site (Hu et al., 2015). Cottonseeds were initially planted in nutrient bowls on a nursery bed on 23 April, 2012 and on 30 April, 2013. Once seedlings had reached the third true leaf stage, uniform seedings were selected to transplant into the field (cotton is routinely transplanted in Yangtze River Valley Cotton Belt in China). The experiment was arranged as a split plot, randomized complete block design with three replications. The main plots were varieties and subplots were K levels. Each plot was 13 × 6.6 m^2 and contained fifteen rows with 0.85 m row spacing and 0.35 m interplant distance. The plant density was 33600 plants ha^{-1}. Treatments consisted of a treatment without K fertilizer (0 kg K_2O ha^{-1}) as the control and two treatments with different K fertilizer application rates (150 and 300 kg K_2O ha^{-1}) using potassium sulphate applied at transplanting. To avoid the influences of sulfur from potassium sulphate, enough ordinary superphosphate (containing 12% P_2O_5 and 12% sulfur) was applied to provide 120 kg P_2O_5 ha^{-1} at the transplanting stage. In addition, 40% and 60% of total N (240 kg N ha^{-1}) fertilizer provided by urea (46% of N) were applied at the transplanting and the initial flowering stage, respectively.

For all plants, the flowering date of the flowers at the 1 st sympodial fruiting position of fruiting branches 7–8th was noted using plastic tags. 4–6 tagged bolls were collected every 7 days from 17 to 45 days post anthesis (DPA). The cottonseeds in the bolls were collected and then separated into two parts. After removing the cottonseed coat, half of the embryos were immediately put into liquid nitrogen and then stored in a −80 °C freezer for subsequent enzyme assays. The other embryos were used for the measurement of oil and K contents. At 45 DPA, the embryos were also used to assay fatty acid composition.

2.2. Embryo K content

Dried embryo samples were sieved with the 1 mm mesh after being ground. 0.3 g samples from each treatment were digested by H_2SO_4-H_2O_2 solution. Then K concentration in H_2SO_4-H_2O_2 solution was determined by a Flame Atomic Absorption Spectrophotometer (TAS-986, PERSEE, China) according to previously defined methods (Yu et al., 2016).

2.3. Oil content and fatty acid composition

Embryo oil concentration was measured according to Luque de Castro and Garcıa-Ayuso (1998) using the Soxhlet extraction method. 1 g dried embryo tissues were tightly wrapped in filter paper and put into a Soxhlet extractor. The oil in the samples was extracted by petroleum ether until the samples reached constant weight. The embryo oil concentration was calculated as 100% × (1- final weight/1 g).

Fatty acid profiling was conducted using gas chromatography according to Liu et al. (2016) with minor modification. 0.1 g dried samples were put into a conical flask. Then 2 mL of 0.5 mol L^{-1} NaOH solutions dissolved in methanol were added before heating extraction at 65 °C for 30 min. Then 2 mL of 15% Boron trifluoride dissolved in methanol was added before heating at 65 °C for 30 min 4 mL heptane was added and the mixture was extracted by heating at 65 °C for 5 min before adding 10 mL saturated NaCl solution. The final supernatant was collected, and fatty acids were identified using a HP 6890 gas chromatograph with a hydrogen flame ionization detector. The separation system was as follows: The chromatograph was fitted with 30 m length Rtx-WAX having 0.25 μm film thickness and 0.32 mm column. Injector temperature was at 260 °C. The column oven temperature was kept at 160 °C for 2 min. Then the temperature increased from 50 to 230 °C with 3 °C/min increments before keeping 10 min. Detector temperature was 270 °C. Peak identifications of fatty acids were conducted through comparing with the corresponding peak values of standard