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Title: Potassium application regulates nitrogen metabolism and osmotic adjustment in cotton (*Gossypium hirsutum* L.) functional leaf under drought stress

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ABSTRACT

To evaluate the role of potassium (K) in maintaining nitrogen metabolism and osmotic adjustment development of cotton functional leaves to sustain growth under soil drought and rewatering conditions, the plants of two cotton cultivars Siza 3 (low-K sensitive) and Simian 3 (low-K tolerant), were grown under three different K rates (K0, K1, and K2; 0, 150, and 300 kg K_2O ha^{-1}, respectively) and exposed to drought stress with 40 ± 5% soil relative water content (SRWC). The drought stress was applied at flowering stage by withholding water for eight days followed by rewatering to a well-watered level (75 ± 5% SRWC). The results showed that drought-stressed plants of both cultivars showed a decrease in leaf relative water content (RWC) and osmotic potential in the functional leaves and developed osmotic adjustment with an increase in the contents of free amino acids, soluble sugars, inorganic K, and nitrate as compared to well-watered plants. In drought-stressed plants, nitrogen-metabolizing enzyme activities of nitrogen reductase (NR), glutamine synthetase (GS), and glutamate synthase (GOGAT) were diminished significantly ($P \leq 0.05$) along with decreased chlorophyll content and soluble proteins. However, drought-stressed plants under K application not only exhibited higher osmotic adjustment with greater accumulation of osmolytes but also regulated nitrogen metabolism by maintaining higher enzyme activities, soluble proteins, and chlorophyll content in functional leaves as compared to the plants without K application. Siza 3 showed better stability in enzyme activities and resulted in 89% higher seed cotton yield under K2 as compared to K0 in drought-stressed plants, whereas this increase was 53% in the case of Simian 3. The results of the study suggested that K application enhances cotton plants' potential for sustaining high nitrogen-metabolizing enzyme activities and related components to supplement osmotic adjustment under soil drought conditions.

1. Introduction

Cotton (*Gossypium hirsutum* L.), being a widely adapted crop, is cultivated in temperate to tropical regions around the globe and experiences sporadic drought and rewetting cycles. It is considered to be a drought-tolerant crop equipped with well-developed stress alleviation mechanisms (Wang et al., 2016). Drought is a key abiotic stress affecting crop growth and productivity in almost 40% of agricultural land around the world, and it is emerging as a challenging threat for future agricultural production (Massacci et al., 2008). Sensitivity of cotton crop towards environmental stresses differs significantly among genotypes, and modern cultivars are characterized as being more vulnerable to drought stress (Ullah et al., 2008). Therefore, improving the acclimation potential of this major fiber crop will be of vital scientific and economic issue in the future.

Drought stress induces alterations in numerous plant metabolic pathways such as glycolysis, photosynthesis, and carbon and nitrogen (N) metabolism (Xu and Zhou, 2005; Abid et al., 2016a). Drought-induced physiological responses might be characterized to minimize water loss despite the increasing external osmoticum, protect subcellular structures, and stabilize internal metabolic processes in plants. It is a dominant dogma in stress physiology that leaf osmotic adjustment during drought stress may contribute to mitigate drought stress by sustaining cell metabolic activities and improving their recovery after rewatering (Morgan, 2003; Parida et al., 2007). Pronounced synthesis and accumulation of compatible solutes such as sugars and amino acids, especially proline, during drought stress mediate osmotic adjustment. Metabolic benefits of the accumulation of osmolytes augment their

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functional significance in stressed plants, and accumulation of these osmolytes varies among cultivars, species, and even among different positions of the same plant (Pilonsmits et al., 1995; Lv et al., 2007). However, the mechanisms of leaf water relations and osmotic adjustment in functional leaves of cotton to maintain metabolic functioning during drought and their contribution in recovery after rewatering are not clear.

Reduced N assimilation and translocation have been reported in plants under drought conditions, and concentration of N varies in plants depending on soil moisture and nutrient status (Xu and Zhou, 2005). The activity of the photo-assimilate apparatus is closely related to N status of leaves since N is an integral part of chlorophyll, proteins, and ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), a key enzyme involved in CO_2 assimilation. Drought stress considerably reduced Rubisco content and chlorophyll content in wheat, and more reduction was observed in nutrient-deficient plants (Abid et al., 2016b). The activities of the key N-metabolizing enzymes, nitrate reductase (NR; EC 1.6.6.1) and glutamine synthetase (GS; EC 6.3.1.2), were also reported to be negatively affected in drought-stressed plants (Xu and Zhou, 2006). NR is an essential enzyme for N assimilation in plants and is also involved in carbon metabolism, but drought can affect its activity (Goel and Singh, 2015). Regulated NR activity is likely to maintain protein and N contents in plant leaves (Singh and Usha, 2003) and hence may contribute in maintaining plant growth under drought stress and recovery after rewatering conditions. GS ensures an organic N supply to the chloroplast for photo-assimilate synthesis (Sibout and Guerrier, 1998). Both GS and glutamate synthase (GOGAT; EC 1.4.7.1) are involved in ammonia assimilation, and alterations in the assimilating pathway under drought stress are also responsible for osmoregulation in plants by de novo synthesis and accumulation of compatible solutes such as proline (Xu and Zhou, 2006). However, information regarding the influence of soil drought stress on the activities of key N-metabolizing enzymes is scant in relation with nutrient application management.

Potassium (K) is one of the essential macronutrients and the most important osmoticum in plants (Wang et al., 2016). Promising evidence is available regarding the role of K in optimizing stomatal conductance, enzyme activation, protein synthesis, gas exchange, photo-assimilate translocation, and stress resistance in different crop species (Wang et al., 2013; Zorb et al., 2014). Information on whether K can facilitate cotton plants to regulate nitrogen metabolism and osmotic adjustment under drought conditions is scarce. Drought events are likely to occur more frequently in the future (IPCC, 2007), and current times present a dire need to focus research efforts on finding and constructing innovative management strategies for ameliorating the consequences of drought stress. Therefore, the objective of this study was to look into the potential of K fertilizer to extenuate and recoup the deleterious effects of drought stress in terms of N metabolism and osmotic adjustment of the cotton functional leaf. The changes induced by K fertilizer in N metabolism and leaf osmotic adjustment in drought-stressed cotton plants may be implicated to formulate operative nutrient management strategies to alleviate the injurious effects of drought stress.

2. Materials and methods

2.1. Plant materials and experimental design

A greenhouse experiment was conducted in 2015 at the Pailou experimental station of Nanjing Agricultural University, Nanjing, China (118°50′E, 32°02′N). Two cotton cultivars, Siza 3 (low-K sensitive) and Simian 3 (low-K tolerant) (Hu et al., 2015), were selected as plant materials. Clear plastic sheets over the green house could be rolled up to allow for the control of sunlight and precipitation. Cotton seedlings were grown in a nursery bed and transplanted at the three true leaves stage into pots (37 cm in diameter and 32 cm in height) filled with 25 kg of well-mixed clay loam soil, collected from the topsoil layer of up to 30 cm depth from the experimental station. The soil chemical properties were as follows: 16.5 g kg^{-1} organic matter, 1.1 g kg^{-1} total N content, 70.8 mg kg^{-1} available N, 23.6 mg kg^{-1}available phosphorus, and 97.3 mg kg^{-1} exchangeable K content. Phosphorus fertilizer was applied at 120 kg ha^{-1} P_2O_5 as a basal dose and N at the rate of 240 kg ha^{-1} in two splits: 40% as basal and 60% at first flower. The K treatments included: 0 (K0) as a control and 150 (K1) and 300 kg ha^{-1} K_2O (K2) were applied using potassium sulphate (K_2SO_4) as a fertilizer. All pots were equally watered up to flower initiation on six to seven fruiting branches. At flowering stage all the pots were divided into two parts. One half of them continued to be watered as before, while the other half experienced water withholding (eight days) until the moisture level reached severe soil drought stress at 40 ± 5% soil relative water content (SRWC) and was then followed by rewatering up to 75 ± 5% SRWC. The soil moisture level of all pots was determined and maintained by collecting soil samples from 0 to 25 cm depth on a daily basis during drought stress treatment at 18:00-19:00 local time with an auger (2 cm in diameter) from different pots of each half. Then the fresh weight of the composite samples was determined, followed by oven-drying at 105 °C for 8 h. Soil water content was expressed as g water g^{-1} dry soil (Liu et al., 2008).

2.2. Sampling and processing

The youngest fully expanded main stem leaves (the fourth leaf from the top, functional cotton leaf) from three different plants were collected on the last day of drought stress (72 days after transplanting) and after 10 days of rewatering. They were then shifted to lab immediately in an ice box for further analysis. Sampled leaves were rinsed with distilled water and divided in two halves by removing the midrib. One half of each leaf was stored in −40 °C for enzyme activity assays of N metabolism (NR, GS, GOGAT) and protein and chlorophyll contents, whereas the other half was dried at 105 °C for 30 min and then to constant weight at 80 °C for further determination of free amino acids, K nitrate (NO_3^-), and soluble sugar contents.

2.3. Leaf relative water content and osmotic adjustment

The relative water content (RWC) of cotton functional leaves was measured according to Barrs and Weatherley (1961). Immediately after sampling, leaves were weighed and then immersed in distilled water for 4 h at room temperature. The leaves were then blotted dry and weighed prior to oven-drying at 80 °C for 48 h. The leaf relative content was calculated using the following formula: RWC (%) = [(FW − DW)/(TW − DW)] × 100, where FW, DW, and TW are the fresh, dry, and turgid weights (weight after the leaf was kept immersed in distilled water for 4 h), respectively.

Osmotic potential was measured using a vapor pressure osmometer (Wescor Vapor 5520, ELI Tech Group Inc., Logan, UT, USA) according to Hummel et al. (2010). Osmotic potential at full turgor (Ψs^{100}) was calculated according to the equation: $\Psi s^{100} = \Psi s \times (RWC/100)$. Then, osmotic adjustment (OA) was calculated as the difference in Ψs^{100} of the control (Ψs_c^{100}) and the stressed plants (Ψs_s^{100}): $OA = \Psi s_c^{100} - \Psi s_s^{100}$

2.4. Chlorophyll content

Frozen leaf samples (0.2 g) were placed for 24 h in a vial with 4 ml of dimethyl sulphoxide for pigment extraction to determine chlorophyll content according to Huang and Zhao (2001). The absorbance of the supernatant was measured by a spectrophotometer (UV-2450, Shimadzu, Japan) at a wavelength of 470 and 648 nm for chlorophyll a and b, respectively. The sum of chlorophyll a and b was used as total chlorophyll contents.