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Waterlogging during flowering and boll forming stages affects sucrose metabolism in the leaves subtending the cotton boll and its relationship with boll weight

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ABSTRACT

The work explored sucrose metabolism in the leaves subtending the cotton boll (SBL) and its role in boll weight after waterlogging in cotton. Results showed that net photosynthesis rate (*Pn*), relative water content, contents of Chlorophyll a and Chlorophyll b, initial ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco) activity and cytosolic fructose-1, 6-bisphosphatase (cy-FBPase) activity decreased with waterlogging in the SBL on fruiting branches 2–3 (FB_{2-3}) and FB_{6-7}. Activities of sucrose synthase (SuSy) and sucrose phosphate synthase (SPS) increased to the maximum up to 6 days of waterlogging then decreased with prolonged waterlogging. Rubisco activation and specific leaf weight increased and gene expressions of *SuSy*, *SPS* and rubisco activase (*RCA*) were all up-regulated with the duration of waterlogging, especially for the SBL on FB_{6-7}. The induction of activity and gene expression of SuSy was most significant indicating its crucial role in sucrose metabolism after waterlogging. For the SBL in the later period of boll development on upper FB_{10-11} and FB_{14-15}, the pattern seemed opposite to that of FB_{2-3} and FB_{6c7} as compensation effect in vegetative growth existed. Correlation analysis revealed that initial Rubisco activity and cy-FBPase activity were the main limitation to *Pn* reduction after waterlogging. Reduction in *Pn*, sucrose transformation rate and initial Rubisco activity directly decrease boll weight in waterlogged cotton. Besides the role in sucrose metabolism after waterlogging, SuSy also had a positive significant correlation with the duration of rapid-accumulation period for seed fiber weight ($P < 0.05$). These findings elucidated mechanisms to waterlogging that affected seed fiber weight, which resulted from alteration in carbohydrates, enzymes and genes.

Introduction

Cotton (*Gossypium hirsutum* L.) is the most important industrial crop in China. However, cotton is sensitive to water waterlogging [1] which has become a major environmental constraint limiting cotton production. This is because the key period for yield formation in cotton is the flowering and boll forming stage [2] and, for the middle and lower reaches of the Yangtze River where cotton is grown, the rainfall mainly occurs in May–October, accounting for 60% of total rainfall over the year. This occurrence of high rainfall in the growing season during this critical stage of yield formation, results in water logging stress to the cotton crop, seriously affecting its growth and yield forming [3,4].

A major component of waterlogging stress is the lack of oxygen available to submerged tissues. Previous studies showed the yield reduction after waterlogging was mainly due to a decrease in boll number [3–5]. As one of the yield components for cotton, boll weight also plays an important role in cotton yield formation. Studies on wheat (*Triticum aestivum*) indicated that lower yield after waterlogging was due to smaller grain weights, rather than to decreases in spike number or grain number per spike [6]. Previous

Abbreviations: WL, waterlogging; CV(%), coefficient of variance; DPA, days post-anthesis; FB, fruiting branches; SBL, leaf subtending the cotton boll or the subtending leaf; MDT, mean daily temperature; MDT_{max}, mean daily maximum temperature; MDT_{min}, mean daily minimum temperature; SFW, seed fiber weight; *Pn*, net photosynthesis rate; RWC, relative water content; Chl a, chlorophyll a; Chl b, chlorophyll b; Rubisco, ribulose-1,5-bisphosphate carboxylase-oxygenase; cy-FBPase, cytosolic fructose-1,6-bisphosphatase; SLW, specific leaf weight mean the weight per cm^{-2} leaf; SPS, sucrose phosphate synthase; SuSy, sucrose synthase; RCA, Rubisco activase.

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studies in cotton waterlogging have looked at boll mass averaged over the entire canopy. But no research has been done on the mass of boll from different positions after waterlogging.

Sucrose and starch are the principal end products of photosynthesis in most plants. Moreover, sucrose is the principal carbohydrate translocated from source to sink tissues and is sensitive to waterlogging stress. Research on *Phragmites communis* [7], mung bean (*Vignaradiata*) [8], and bottomland hardwood [9] showed that the root of tolerant cultivars had more carbohydrates. For the susceptible cultivar, it was reported that phloem transport of photosynthates was blocked [10], and the demand for sucrose loading was lowered. This may lead to an accumulation of starch in the leaf chloroplasts [11,12]. The pattern of carbohydrates also differs for different durations of waterlogging. Research on luffa (*Luffa cylindrica* Roem,) and bitter melon (*Momordica charantia* L.) showed the starch content of roots significantly decreased during the early stage of waterlogging. After 6 days of waterlogging, starch content of luffa root increased, whereas that of bitter melon was still lower than non-waterlogged plants [13]. In the early stage of waterlogging, the total soluble sugar content (sucrose and hexose) was 3.5–4.0 times higher than that of untreated plants for tolerant and susceptible species, and it decreased to untreated plants levels with the duration of waterlogging, which was observed in the reports of Perata [14], Barta [12] and Castonguay [15]. Sucrose could sustain Embden–Meyerhof–Parnaspathway (EMP) by SuSy, enhancing the ability to resist the effects of waterlogging [16]. Research on Pondweed (*Potamogeton distinctus* A. Benn.) turions clearly showed enhancement of starch consumption in turions during anaerobic growth of stems of pondweed, whereas the sucrose content in turion cells decreased rapidly in the early stage of anaerobic growth but then remained constant after the enhanced growth had started, suggesting active sucrose metabolism [17].

In the Calvin cycle and sucrose synthesis processes, sucrose metabolizing enzymes have been studied extensively. Rubisco is the key and rate-limiting enzyme in the Calvin cycle. SPS, a key regulatory enzyme involved in carbon partitioning between sucrose and starch in leaves [18], is often closely correlated with the rate of sucrose export in source tissues It catalyzes the penultimate step in sucrose synthesis, and shares control of this pathway with the first committed step catalyzed by cytosolic FBPase [19]. A recent study about SuSy with orchid (*Oncidium goldiana*) suggested that its crucial function in plant metabolism was mainly sucrose breakdown and energy provision [20]. Enzymes related to sucrose metabolize were all affected by waterlogging. Research on bald cypress (*Taxodium disffchum*) seedlings found that Rubisco activity decreased for waterlogged plants [21]. The activation level of Rubisco in flooded bitter melon (*Momordica charantia*) increased above the control value after 1 day of flooding and subsequently declined to a lower level, and the Rubisco activity decreased to 59% of non-flooded bitter melon after 7 days of waterlogging [22]. The *Pn* decrease for waterlogged rice (*Oryza sativa*) was mainly due to reduction in activities of Rubisco and FBPase [23]. The activities of SuSy, SPS, acid invertase were all enhanced in anaerobic conditions, and the induction of the activity of sucrose synthase was most significant, suggesting that sucrose synthase plays an important role in sucrose metabolism in pondweed turions growing in anaerobic conditions [17]. Other research has shown that the catalysis of sucrose cleavage by invertase in roots was replaced by SuSy under waterlogging [24–28]. Thus, sucrose cleavage catalyzed by SuSy in waterlogging condition has advantages over invertase in waterlogging stress, playing a crucial role in providing an adequate sugar supply during anoxic stress [29,30].

Genes encoding for enzymes involved in sugar and sugar-phosphate metabolism, including glycolysis, were generally upregulated during hypoxia in rice (*Oryza sativa*), *Arabidopsis thaliana* and poplar (*Populus x canescens*), with only an exception for invertase [31]. These genes were down-regulated upon the various hypoxic stress treatments, as well as genes encoding enzymes of amino acid metabolism which were generally down-regulated [31]. Other researches also showed an induction of SuSy expression in waterlogging condition [32]. Gene expression studies done using RT-PCR in 24 h waterlogged mung bean (*Vigna radiata*) showed enhanced expression of *SuSy* in the roots of a tolerant genotype, while in susceptible genotypes there was no change in expression level in control or treated plants [8]. However, studies on the molecular responses to soil waterlogging in cotton have been comparatively scarce, with the first such study by Christianson (2010) was done at the two-leaf stage [33]. He found that SuSy expression was up-regulated by 12 times in the root while it was not significantly up-regulated in the leaf compared to that of non-waterlogged cotton.

Changes in expressed protein, gene transcription and metabolite levels have been studied in responses to waterlogging stress in rice, mung bean, and wheat. However, little research has been done on sucrose metabolism in the SBL on different main-stem fruiting branches after waterlogging in cotton, and the metabolism affected boll weight from alteration in carbohydrates, enzymes and gene expressions in the SBL has not been answered yet. The objective of our research, therefore, was to investigate the physiological mechanism for changes of seed fiber weight per boll after waterlogging during the flowering and boll forming stage on the basis of sucrose metabolism in the SBL on different main-stem fruiting branches.

Materials and methods

Plant materials and growth conditions

Experiments were conducted in the summer seasons of 2011 and 2012 in ponds with transparent waterproof top at the experimental station of Nanjing Agricultural University, located at Nanjing (N 32°02′ and E 118°50′), China. Cotton seeds (cv. Siza 3) were planted on 8 April 2011 and 2012. Individual healthy and uniform seedlings with three true leaves were transplanted into ponds (4 m in length, 4 m in width and 1.5 m in height) containing yellow-brown soil (Dystrudept) which was collected from (0 to 30) cm topsoil layer from the experimental station. Each pond was consisted of 5 rows, the row and plant spacing was 75 × 25 cm in each year.

Experimental designs and treatments

The experiment was conducted in a completely randomized design with five levels of soil water managements. Three ponds were assigned at random to each treatment.

Five soil water treatments were established during the flowering and boll forming stage: (i) a well-watered control (WL_0) with soil relative water content maintained at 70–80% of water capacity during the experiment; and (ii) four waterlogging treatments (WL_3, WL_6, WL_9, WL_{12}) with a 1–2 cm water layer maintained above the soil surface, until the evening of the 3rd, 6th, 9th and 12th day when water was removed by opening holes at the bottom of pools.

Harvest of samples

White flowers at main-stem sympodial fruiting branches 2–3, 6–7, 10–11 and 14–15 of the cotton plants were tagged. Bolls were mapped by main-stem node and sympodial branch fruiting position. The first sympodial position closest to the main stem was designated fruiting position 1-boll, and successive boll positions were designated fruiting position 2-boll and fruiting position 3-boll. 10–15 tagged position 1 bolls and the SBL on FB_{2-3}, FB_{6-7},