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Combined elevated temperature and soil waterlogging stresses inhibit cell elongation by altering osmolyte composition of the developing cotton (*Gossypium hirsutum* L.) fiber

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ABSTRACT

Soil waterlogging events and high temperature conditions occur frequently in the Yangtze River Valley, yet the effects of these co-occurring stresses on fiber elongation have received little attention. In the current study, the combined effect of elevated temperature (ET) and soil waterlogging (SW) more negatively affected final fiber length (reduced by 5.4%–11.3%) than either stress alone by altering the composition of osmotically active solutes (sucrose, malate, and K^+), where SW had the most pronounced effect. High temperature accelerated early fiber development, but limited the duration of elongation, thereby limiting final fiber length. Treatment of ET alone altered fiber sucrose content mainly through decreased source strength and the expression of the sucrose transporter gene *GhSUT-1*, making sucrose availability the primary determinant of final fiber length under ET. Waterlogging stress alone decreased source strength, down-regulated *GhSUT-1* expression and enhanced SuSy catalytic activity for sucrose reduction. Waterlogging treatment alone also limited fiber malate production by down-regulating *GhPEPC-1 & –2*. However, combined elevated temperature and waterlogging limited primary cell wall synthesis by affecting *GhCESAs* genes and showed a negative impact on all three major osmotic solutes through the regulation of *GhSUT-1*, *GhPEPC-1 & –2* and *GhKT-1* expression and altered SuSy activity, which functioned together to produce a shorter fiber length.

1. Introduction

Heavy rainfall events are occurring with greater frequency and are accompanied by high temperature from May–October in the middle and lower reaches of the Yangtze River, China; furthermore, global temperatures are expected to increase by 2–4 °C in the coming decades [1,2]. Thus, it is expected that co-occurring waterlogging and high temperature stress will have pronounced, negative effects on crop production in this region.

A number of researchers have reported the negative impacts on cotton yield and fiber quality in response to either high temperature [3–5] or waterlogging [6–8] stresses individually, whereas the combined effects of these two stresses are not well studied. For example, it was reported that temperature affected fiber quality more than other meteorological factors [9]. Liakatas showed that fiber length, strength, uniformity and micronaire value were affected by high temperature, especially high daytime temperature [10]. Gipson found the initial stage of fiber elongation was more sensitive to high night temperature [11], and the optimum night temperature range for fiber elongation was between 15 and 21 °C [12]. Waterlogging also showed negative effects on fiber length and strength [13] by changing sucrose metabolism enzyme activities and relative gene expression thereof [14]. The co-effects of high temperature and waterlogging decreased fiber length and increased fiber micronaire value [15], and high temperature exposure was found to decrease fiber length even after removal of the waterlogging stress [16].

As the most important agricultural textile commodity, upland cotton (*Gossypium hirsutum* L.) fiber has been a good model for exploring cell elongation because of its distinct developmental stages including initiation, elongation, secondary cell wall

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biosynthesis and maturation [17,18]. Fiber usually elongates rapidly from 0 to 16 DPA (days post anthesis), during which the primary cell wall is deposited [19]. At about 14–16 DPA, the fiber elongation rate begins to slow down with the start of secondary cell wall synthesis, and stops usually at approximately 20–24 DPA [20,21]. It is widely accepted that cell turgor pressure [20,22] and many other factors play important roles in the fiber elongation process. For example, cell turgor is impacted by the transport and metabolism of osmotically active solutes [22,23], the energy required for the transmembrane transport of osmolytes [24], expression of channel proteins, and expression of genes encoding proteins required for cell wall loosening [23,25,26]. Additionally, plasmodesmata (PD) were reported as gateways controlling the generation of fiber cell turgor, which are usually closed between 10 and 15 DPA to block macromolecules from transferring outward. With the high expression of sucrose transporter (SUT) and K^+ transporter (KT), osmotically active solutes are highly accumulated in fiber, which leads to increased fiber cell turgor and rapid fiber cell elongation [27]. Both a higher rate and a longer duration of fiber elongation would allow a fiber cell to elongate to a greater final fiber length, which is an important fiber quality characteristic in cotton [20,28]. Soluble sugars, malate, and potassium (K^+), together account for about 80% of sap osmolality inside the fiber, and are regarded as the major osmotically active solutes [22,29,30]. Previous studies have shown that soluble sugars and K^+ were imported into the cotton fiber from the phloem of the seed coat, whereas malate was synthesized locally through the initial activity of phosphoenolpyruvate carboxylase (PEPC) in fiber cytoplasm [20,24]. Sucrose has been considered the main form of soluble sugar transferred into the fiber and its transport is mediated by sucrose transporter (SUT), while K^+ transporters (KT) import K^+ into elongating fibers. Besides, pectin precursors have also been reported to play a role in affecting root hair elongation in *Arabidopsis* [31] and controlling the cell extensibility and elongation in ripening fruits [32].

Most researchers focused on the relation between fiber elongation and sucrose metabolism [33,34], because sucrose supplies energy for fiber elongation in addition to its role as a major osmotically active solute [14]. In elongating fiber cells, sucrose was mainly degraded by sucrose synthase (SuSy, E.C. 2.4.1.13) and vacuolar invertase (VIN, E.C. 3.2.1.26) [23,35]. Ruan showed that fiber elongation was affected by suppression of *SuSy* gene expression in transgenic cotton (*Gossypium hirsutum*) ovules [35], and the decreased fiber length was related to the lower SuSy activity under low light [34]. Additionally, VIN was reported to play a role in early fiber elongation by osmotic regulation of vacuole enlargement [23]. Sucrose is the form of carbon universally used for long distance carbon transport in vascular plants employing the apoplastic pathway of phloem loading, and its transporters have vital roles in plant growth and development. Many types of sucrose transporters have been discovered since *SoSUT-1* was first identified by Riesmeier et al. using a screening system with yeast mutant [36,37]. Ruan et al. identified the essential role of sucrose transporter *GhSUT-1 in the elongating cotton fiber* [20]. *Moreover, sucrose can also be degraded to uridine diphosphate glucose (UDPG), which is used to synthesize cellulose and callose during the secondary cell wall biosynthesis* [38]. *The synthesis of cellulose is regulated by cellulose synthase, while callose is synthesized by callose synthase (mainly regulated by GhCalS5)* [39,40]. Li [41] reported that *GhCESA1* and *GhCESA8* played an important role during the secondary cell wall biosynthesis, while *GhCESA3*, *GhCESA5* and *GhCESA6* mainly worked for primary cell wall synthesis, and some recent data indicated the possible association of SuSy and CESA complexes for high cellulose production [42]. Due to the importance of K^+ as an osmoregulator, field studies showed that soil K deficiency reduced K concentrations in the cotton fiber cell [43], which led to decreased final fiber length [44–46]. Similar to sucrose, K^+ in the fiber cell is mainly transferred from the underlying seed coat cells by K^+ transporters [20]. *GhKT-1*, encodes a cotton K^+ transporter, and may function along with *GhSUT-1* for the maintenance of fiber cell turgor pressure required to drive fiber elongation [20,25,47]. Unlike sucrose and K^+, malate was synthesized within fiber cells through phosphoenolpyruvate carboxylase (PEPC) [48]. Li et al. indicated that high activity of PEPC can affect cotton fiber elongation, likely through the expression of *GhPEPC*-1 and −2 genes [48]. When considering the impact of waterlogging and high temperature stress on fiber development and carbon dynamics, it is also important to document the impact of these co-occurring stresses on source strength. In cotton, greater than 60% of the carbohydrate requirement for boll development comes from the leaf that subtends the boll on a fruiting branch [49]. Thus, abiotic stresses (i.e. high temperature) that negatively impact the photosynthetic rate of the subtending leaf also limit the carbohydrate content of the immediately adjacent reproductive structure [50] and would be expected to limit carbohydrate supply to the developing fiber.

Previous studies mainly addressed the effects of high temperature or waterlogging individually, or focused on fiber elongation without considering how the three major osmotically active solutes respond to these abiotic stresses during fiber development. Studies that provide a comprehensive assessment of waterlogging and high temperature stress effects on fiber development in relation to source strength and osmolyte impacts on the developing fiber are to our knowledge, nonexistent. In the current study, it was hypothesized that: (1) elevated temperature (ET) and soil waterlogging (SW) would exhibit a more pronounced negative effect on fiber elongation and final fiber length than either stress in isolation; (2) that fiber elongation responses to these combined stresses will be related to source limitations, alterations in osmotically active solute content and related enzymes and genes participating in fiber elongation. Consequently, our objectives in the current study were to (1) quantify the co-effect of elevated temperature (ET) and soil waterlogging (SW) on fiber elongation and final fiber length; (2) to characterize the impact of ET, SW and combined ET and SW on the photosynthetic rate of the subtending leaf and fiber osmotically active solutes; and (3) identify the key enzymes and gene isoforms participating in fiber elongation under combined ET and SW conditions.

2. Materials and methods

2.1. Plant material and growth conditions

Experiments were conducted in plots (each one is 4 m in length, 4 m in width and 1.5 m in height) with a transparent waterproof top for three cotton growing seasons from 2013 to 2015 at Pailou Experimental Base (118°50′E, 32°02′N), Nanjing Agricultural University, Nanjing, China. The yellow brown soil used in plots was collected from the upper 30 cm of topsoil layer at the experimental base. The soil contained 69.5, 74.3, and 77.8 mg kg^{-1} alkali-hydrolysable nitrogen (N); 17.1, 20.3, 18.5 mg kg^{-1} phosphorus (P) and 101.4, 107.8, 112.5 mg kg^{-1} potassium (K), from 2013 to 2015, respectively. Cotton seeds (cv. Siza 3) were planted on 7 April in all growing seasons, and individual uniform seedlings with three true leaves were transplanted into ponds. Each pond contained 5 rows, and the inter-row and intra-row plant spacing was 75 × 25 cm, respectively.

2.2. Experimental designs and treatments

The experiment was conducted as a completely randomized and full factorial design with two temperature regimes and three levels of soil water management. Both elevated temperature regime